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THE NEW PHYTOLOGIST

84693



PRINTED IN GREAT BRITAIN

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## TORSIONS AND THEIR ANALYSIS

By R. SNOW

*Fellow of Magdalen College, Oxford*

(With 9 figures in the text)

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## 1. INTRODUCTION

HOW do petioles, flower stalks, dorsiventral stems and other dorsiventral organs carry out the torsions by which they orientate themselves to light and gravity? A very full and valuable discussion of this rather neglected problem, with references to earlier work, has been given by Rawitscher (1932), and some experiments by Staub (1934) will be discussed at the end of the present paper. Rawitscher concludes that these torsions cannot in general be explained as due to two successive curves in different planes: for though two such curves *can* cause torsions, yet many organs carry out torsions while remaining practically straight. He rejects also various other early attempts to explain these torsions, and concludes (pp. 201 seq.) that the only adequate explanation is that when the organ is stimulated, its cells elongate obliquely, in directions which form helices round the long axis of the organ, instead of elongating parallel to that axis. It must further be supposed that the helices may be either right-hand or left-hand according to the direction in which the stimulus is acting, since in nature the organs always twist into the correct orientation to the stimulus by the shorter way round. This conclusion agrees with that of Frank (1870).

The question next arises whether this puzzling oblique elongation of the cells can be analysed and shown to result from simpler processes, and so be made easier to understand. Rawitscher (p. 203) considers that such an analysis can be suggested, and he bases it on certain ideas by which Sachs (1879) tried to make plagiotropism easier to understand, and which were in turn derived and modified from an earlier hypothesis of Stahl (1877). Sachs suggested that a plagiotropic and dorsiventral organ, like the thallus of a liverwort, might be considered as if made up of a number of orthotropic 'elements' of some kind, which normally stand erect. These elements, which may be thought of most easily as being like little seedlings, must react to stimuli of light and gravity, like shoots in their upper parts and like roots in their lower parts. If now the whole plagiotropic organ is stimulated by light or gravity from one side, its response can be understood as resulting from the independent orthotropic reactions of the 'elements' within it.

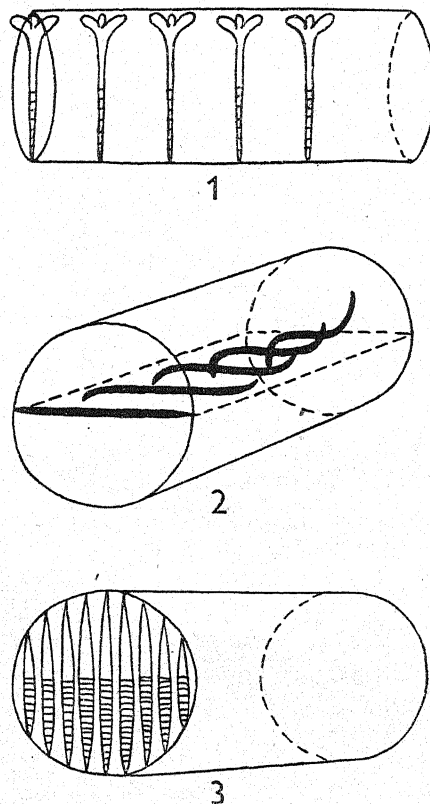
Rawitscher starts with a similar idea in trying to analyse the torsions, and first suggests that a dorsiventral and normally horizontal organ, such as a dorsiventral shoot, may be thought of as containing a row of erect orthotropic elements standing in the dorsiventral plane of the organ. These are represented in Fig. 1 as if they were little seedlings. If now the whole organ is rotated through, say,  $90^\circ$ , as in Fig. 2, the elements within it will tend to curve up at their tips and down at their

bases by geotropism (Fig. 2), and so, since the whole organ is fixed at one end and free at the other, they will force it to twist back into its normal orientation. This hypothesis might be developed further, in accordance with the ideas of Stahl and Sachs, by supposing that each transverse section of the whole organ contains not one but many orthotropic elements, as in Fig. 3. Naturally the curvatures of the orthotropic elements would be due to differences between the rates of growth on their two sides.

However, Rawitscher, who is not satisfied with this first hypothesis, prefers to modify it in a different way, and so suggests what is in effect a second hypothesis, which will be discussed in § 8. He also considers that the supposed changes in the rates of growth in planes transverse to the long axis of the whole organ, such as cause the curvatures of the orthotropic elements, will divert the normal elongation of the organ which is going on at the same time, and cause its cells to elongate obliquely: and in this way he is able still to regard oblique elongation as being the immediate cause of a torsion, although according to his analysis the oblique elongation depends in turn on the transverse growth changes which are the primary growth responses to the stimulus.

In any case all these hypotheses have in common the point that the primary growth responses to the stimulus are supposed to be changes which take place in each transverse section of the whole organ and in directions transverse to its long axis; and they will therefore be called the 'transverse growth' hypotheses.

Instead of these hypotheses it might be suggested that the oblique elongation of the cells, to which the torsion is attributed, is primary and results directly from the properties of the organ. On this view, which is that of Schwendener & Krabbe (1892), it would have to be supposed that when a horizontal leaf, for example, is illuminated from, say, the left as seen from its tip, then the cells of the petiole react by elongating obliquely in directions which form helices running in the one sense (right-hand) round the central axis of the petiole; whereas if the leaf is illuminated from the right, then the cells elongate along left-hand helices. For these reactions very complex properties would be needed in the organ concerned, but perhaps not much more complex than for the lateral geotropism of winding shoots, which is now well established. An hypothesis of this kind will be called for short an 'oblique elongation' hypothesis, and



Figs. 1, 2, 3 are explained in the text. 1 and 2 are copied from Rawitscher (1932).

will be contrasted with the 'transverse growth' hypotheses. For on the latter the oblique elongation of the cells, though it does take place, is not the primary reaction.

Now it is possible by a simple experiment to exclude for any given organ either the one kind of hypothesis or the other, and such an experiment on various organs will be reported in the present paper. An organ which responds by active torsions to stimuli of light or gravity will be called photostrophic or geostrophic, in agreement with Czapek (1898, p. 273).

## 2. PRINCIPLE OF THE EXPERIMENT

If a negatively geotropic shoot is placed horizontal and fixed at its tip, but left free at its base, it curves so as to point its basal end upwards, and so orientates itself the wrong way up. Correspondingly, if one rotates through  $90^\circ$  a geostrophic dorsiventral organ, such as a leaf or a lateral shoot, and if one fixes it at its tip but leaves it free at its base, will it twist the same way as when fixed at the base, or the opposite way? On the 'transverse growth' hypothesis (Figs. 1, 2) it should still twist the same way when fixed at the tip, by which is meant that it should bring the correct face uppermost by twisting the shorter way round, through  $90^\circ$ . For on this hypothesis the changes in rates of transverse growth in each transverse section of the organ are primary, and must therefore take place in the same way whichever end is fixed: and in doing so they will make the organ twist the same way, though the helix which will be formed in consequence by any previously straight longitudinal line on its surface will run in the opposite sense if the tip is fixed instead of the base. But on the 'oblique elongation' hypothesis the primary response is that the cells elongate obliquely in directions forming parallel helices of which the sense is determined by the direction of the stimulus. So the cells must do this just the same whichever end of the organ is fixed; and if they elongate along helices of the *same* sense when the tip of the organ is fixed instead of the base, then the organ will twist the *opposite* way.

One can easily see that this is so if one makes tests with a dry half pea or bean pod, which coils itself (by oblique contraction of dry cells) into a helix of which the sense is structurally determined. If now one straightens out the half-pod by pulling its ends, and if one then lets go one end, the half-pod inevitably coils itself again into a helix of the same sense whichever end one lets go: but the direction in which it rotates in so doing is opposite according as one lets go the one end or the other.

Of course the explanation is that the direction of the force due to the oblique elongation or contraction depends on the end which is held firm. If then one resolves this force into one component parallel to the long axis and another tangential to the axis, it is the latter which causes the torsion, and its direction also is opposite according as one end of the organ or the other is held firm. So the expectation is quite different from what it is on the 'transverse growth' hypotheses, according to which the direction of the tangential force must be the same whichever end is held firm.

On the 'oblique elongation' hypothesis, therefore, a geostrophic organ placed



on one side should twist in the opposite direction if fixed at the tip instead of the base, and so should bring its normally lower face to the top. It should then continue to twist, if still capable of doing so, and should pass through the inverted position as being presumably one of labile equilibrium, and come to rest in the normal position, having reached it by twisting the long way round, through  $270^{\circ}$ .

It seems not to have been noticed that this simple experiment should give opposite results on the two hypotheses. Indeed, Rawitscher 1932 states (p. 203), without giving details, that an organ fixed at the tip twists the opposite way to the normal, although if it does so this is inconsistent with his own hypothesis. For convenience an organ which twists in such a direction that it approaches the correct orientation to the stimulus by the shorter way round will be said to twist in the 'normal' direction, and the opposite direction of twist will be called the 'abnormal' direction. In natural conditions strophic organs always twist in the 'normal' direction.

### 3. METHODS

The experiments were performed on the young growing pinnate leaves of *Spiraea Aitchisoni*, ash and a few other woody species, and also for comparison on leaves of *Phaseolus multiflorus* and leaflets of *Wistaria sinensis*, which respond by movements of pulvini. The leaves were cut off at the base and arranged horizontally or nearly so, but 'on edge'—that is, after being rotated through  $90^{\circ}$ —so that they were stimulated by gravity. They were fixed at the required points, usually by being attached to glass weights with strips of plasticine. With the growing leaves the plasticine was put either round the basal end of the petiole or else round a zone of the rachis near its apical end, but not quite at the end since the end part was too thin and weak. So when the attachment was near the apical end there usually remained two pairs of leaflets above it. With the leaves that had pulvini the plasticine was applied immediately below or above the reacting pulvinus. The leaves were supplied with water in one of two ways: either the base of the petiole was depressed a little so that it dipped into a film of water on a plate, while the rest of the leaf was in damp air, the whole being under a bell-jar, or else the whole leaf was submerged in a basin of water. The second method usually seemed the better.

The basin with the submerged leaves was placed in a greenhouse in which the light came fairly evenly from all sides, the direct sunlight being partially screened by a blind on one side. In order further to diminish any complications from one-sided stimulation by light in directions other than that of gravity, the basin was turned at intervals, so that the bases of the leaves were kept pointing towards the sun. So the strophic stimulus of light acted mainly from above, in the same direction as gravity. In any case it seemed that with the leaves of these species the strophic effect of light was much less than that of gravity. The leaves in damp air usually needed to be shaded completely from direct sunlight. The leaves other than those with pulvini responded only while still growing, and they were taken when at about one-third of their final length. All the leaves responded best in sunny weather.

## 4. RESULTS WITH GROWING PINNATE LEAVES

The growing pinnate leaves which were placed 'on edge' and attached by a zone near the apical end of the rachis all twisted in the normal direction in the part of the rachis below the attachment, just as they did in the short part above the attachment, except for some that failed to twist at all. Those which were attached at the base as controls naturally twisted in the normal direction also, and they too did so by means of the rachis.

Thus out of fourteen leaves of *Spiraea Aitchisoni* attached near the tip, one failed to twist, and thirteen twisted in the normal direction below the attachment, through angles ranging from 10 to 50°, with a mean of 27°. Seven of these twisted 30° or more.

Also out of seventeen leaves of ash attached near the tip, eight failed to twist, and nine twisted in the normal direction below the attachment, through angles ranging from 30 to 70°, with a mean of 44°. From these results a few leaves have been omitted which failed to twist, probably because they were set up in dull rainy weather. The torsions in these two species sometimes reached their maximum in 7 or 8 hr. of a sunny day, but more often they needed 24 hr., and sometimes even more. That the torsions did not reach 90° was probably due to some lack of vigour in detached leaves.

A few leaves of other species were tested similarly. Four leaves of *Wistaria sinensis* twisted in the normal direction below the attachment, through 65, 40, 40 and 30° in 6 hr. Two leaves of *Koelreuteria paniculata* twisted similarly below the attachment, through 30 and 25° in 24 hr., and three of walnut through 20, 15 and 15° in 24 hr. *Wistaria* seems to react the best of all.

The above results exclude for these five species the hypothesis that oblique elongation of cells is primary, and they are consistent with a 'transverse growth' hypothesis.

## 5. THE QUESTION OF COMPLICATIONS DUE TO CURVATURES

It may, however, be suggested that the torsions made by the growing pinnate leaves placed on edge were the effects of successive curves in two different planes due to negative geotropism and epinasty: for by placing a leaf on edge one separates these two factors which previously were balanced against each other.

Now actually of the nine ash leaves which twisted below the attachment only one made a slight negative geotropic curve, though most of them made quite strong epinastic curves, beyond those which they had made previously. Also of the *Spiraea* leaves which twisted only a few made slight negative geotropic curves, and the majority none at all. The leaves of walnut, *Koelreuteria* and *Wistaria* made no negative geotropic curves, while those of *Koelreuteria* and two of those of *Wistaria* made no epinastic curves either, and had not been curved by epinasty at the start, or scarcely at all. Thus most of the leaves which twisted did not at any time make curves in two planes, and some of them were practically straight all the time.

But even when there were curves in two planes, did these curves tend to cause torsions in the directions actually observed? If one takes a young pinnate leaf with an epinastic curve, and if one imposes on it a curve in the transverse plane by

holding one end and pressing the other end to one side, one can easily see that the leaf twists in a direction such that the convex side of the second curve moves round by the shorter way towards the concave side of the first curve. Now in the above experiments the leaves were mostly a little curved by epinasty at the start. So in those few which curved slightly by negative geotropism when placed on edge, this second curve must have tended to make them twist in the opposite direction from that in which they actually did twist.

#### 6. EXPERIMENTS ON LEAVES WITH PULVINI

For comparison with the experiments on growing pinnate leaves, corresponding experiments were performed on full-grown or nearly full-grown leaves and leaflets which respond by movements of pulvini. The leaves tested were mostly the simple primary leaves of seedlings of *Phaseolus multiflorus*, which respond by means of a pulvinus at the top of the petiole just below the leaf blade. Occasionally the terminal leaflets of later leaves of the same plants were used instead, after removal of the two lateral leaflets: these leaflets also respond by a pulvinus just below the blade. The leaves were arranged 'on edge' and kept submerged, being attached to weights by loops of plasticine placed either just below or just above the active pulvinus. The weather was warm and sunny.

Out of fourteen leaves or leaflets of *Phaseolus* which were attached just above the active pulvinus, one failed to twist and in thirteen the pulvini twisted in the *abnormal* direction through angles ranging from 8 to 35°, the mean being 19°. In all but three of the leaves these angles were reached on the first day, after periods of from 7 to 10 hr. Only one of these leaves made a slight negative geotropic curve.

In three other leaves which were attached just below the active pulvinus, this pulvinus naturally twisted in the normal direction, the angles reached being 45, 25 and 15° after 9 hr.

It also seemed of interest to test the secondary pulvini of *Wistaria sinensis*, which orientate the leaflets, since the growing main rachis in this species amongst others had been found to twist in the normal direction when fixed apically: (the main pulvinus does not twist, or not much). So leaves of *Wistaria* with leaflets expanded but not fully mature were cut up into pieces of rachis each carrying one pair of leaflets. The pieces were arranged under water with the leaflets 'on edge', and were attached to weights either just below the pulvini, by a loop of plasticine placed round the main rachis just below them, or else just above a pulvinus by plasticine placed round the basal part of the blade of a leaflet.

After 8 or 9 hr. the pulvini of six leaflets which were attached above them had all twisted in the abnormal direction, the mean angle of twist being 12.5°, and the extremes 10 and 15°.

Six pulvini which were attached below had all twisted in the normal direction, the mean angle being 21°, and the extremes 15 and 25°. None of these leaflets made more than a very slight negative geotropic curve, if any.

Thus pulvini, in the two species tested at least, respond, when the point of attachment is above them (or more strictly 'distal' to them) by twisting in the abnormal direction, and so in the opposite way to the growing pinnate leaves; and

this excludes any kind of 'transverse components' hypothesis for them, and is consistent with an hypothesis of oblique elongation, or rather expansion.

#### 7. A SEARCH FOR ANATOMICAL EVIDENCE

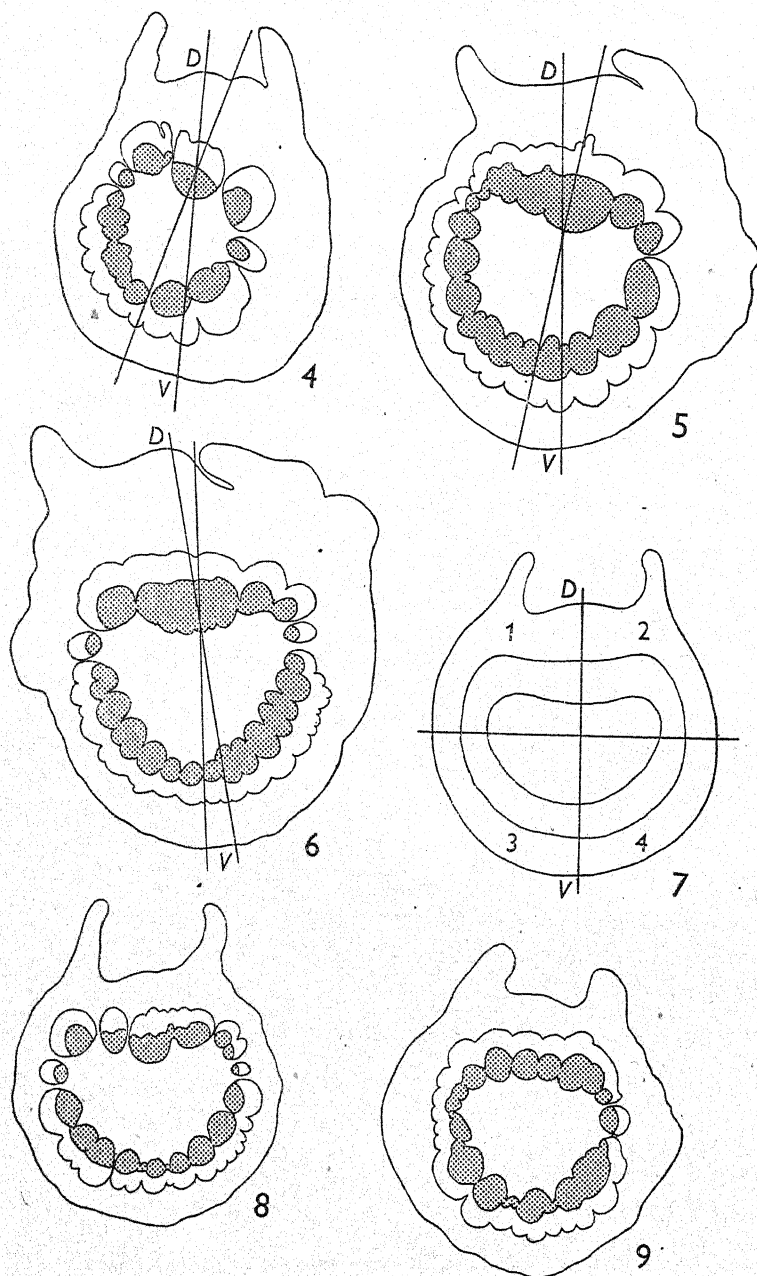
A search was also made for anatomical evidence bearing upon the 'transverse growth' hypothesis. In transverse sections of leaves of a weeping ash, which had twisted very sharply in response to gravity, nothing unusual was seen. So since any anatomical changes might have been 'used up' in causing torsions, arrangements were made to stimulate pinnate leaves by gravity for long periods, while they were mechanically prevented from twisting. For this purpose a shoot of common ash, still attached to the plant, was bent down to the horizontal, and a pair of young leaves which were now horizontal and 'on edge' were pressed forwards so that they met beyond the shoot apex, and were then bound firmly together with the dorsal surfaces of their petioles and rachises in contact, by means of a number of bast ties at fairly close intervals. Also young leaves on a plant of *Spiraea Aitchisoni*, which were horizontal and 'on edge', were bound similarly to nearly opposite older leaves. After several days or longer the leaves were cut off and fixed in alcohol while still bound together.

Some of these leaves which had been left to be stimulated for 6 or 7 days showed nothing unusual in transverse sections of the rachis, but a young leaf of *Spiraea* which had been left for 17 days showed changes which were striking but difficult to interpret. Figs. 4-6 show transverse sections through the apical, middle and extreme basic levels of a young 'leaf internode'—that is, of a portion of the rachis between two pairs of leaflets. The sections are drawn with the dorsal face uppermost, and the side which was on top during the stimulation is on the left. The sections were made through the short zones, only 2 or 3 mm. long, between the bast ties: the zones constricted by the ties had not changed so much. Figs. 8 and 9 show for comparison sections through the apical part and the extreme base of an 'internode' of a normal leaf.

It can be seen that the sections of the stimulated leaf shown in Figs. 4 and 5 are asymmetric, the cortex having apparently shifted round a little in the normal direction relatively to the stele. To make this clear the median dorsiventral planes of the stele and of the whole petiole are marked with straight lines. Fig. 6 is less asymmetric.

It can also be seen that in Figs. 4 and 5 the cortex on the side which was the lower during stimulation (that of quadrants 2 and 4 in the diagram, Fig. 7) has greatly enlarged—in Fig. 5 more strongly in quadrant 2—whereas in Fig. 6, showing a section from the base of a leaf internode, it is the cortex of the dorsal quadrants, 2 and 1, which has enlarged, as is clear on comparison with Fig. 9. This curious and unexplained difference between the reaction of the extreme base of a leaf internode and that of its other levels was found in the neighbouring leaf internode also. In the enlarged regions of cortex all the cells were very much enlarged. The enlargement of the cortex of the lower side may possibly have been due to negative geotropism rather than to the geostrophic stimulus. But as against this, the negative geotropism of these leaves when 'on edge' is feeble, and it anyhow





Figs. 4-9 are explained in the text. They are drawn under projector, and  $\times 23$ , except fig. 7 which is diagrammatic. The xylem is shaded, and the next line outside it shows the outer edge of the fibres round the phloem.

cannot account for the fact that at the base of each leaf internode it was quadrants 2 and 1 that enlarged.

In tangential sections through the side that had been the lower it was seen that in the enlarged cortex the originally longitudinal rows of cells had been thrown into wave-like folds, and these looked as if they were due to the mechanical constraint which had prevented the cortex from elongating. The folding was stronger in quadrant 2 than 4, and the stele was completely unaffected. Whether the folding had anything to do with the strophic stimulation is uncertain.

## 8. DISCUSSION

The results of the experiments with growing pinnate leaves of woody plants, of which the rachis twisted in the normal direction when fixed apically instead of at the base, exclude for the species tested the hypothesis that an oblique elongation of cells is primary, and are favourable to a 'transverse growth' hypothesis for the reasons given in § 2. They also make it seem probable that the pinnate leaves of most other woody species would respond similarly. But in the leaves with pulvini, the pulvini twisted in the *abnormal* direction when fixed apically, and it therefore seems that their torsions must be brought about in quite a different way from those of the growing pinnate leaves, even apart from the difference between movements due to growth and those due to changes of turgor. The responses of the pulvini are consistent with an hypothesis of oblique expansion of cells.

But this is not the end of the complication: for it cannot safely be concluded that even growing leaves of other kinds would all react similarly to the pinnate leaves of woody plants. Indeed some incomplete experiments on simple leaves of herbs of Labiatae, which twist by means of the petiole, have already suggested that these follow the same rule as the pulvini.

However it is still of interest that at least one group of leaves does respond in the sense to be expected on the 'transverse growth' hypotheses, and for these leaves therefore these hypotheses must be taken seriously. A few comments may here be made on the comparative merits of the first and second of these hypotheses set out by Rawitscher 1932. The first hypothesis, described in § 1, has the advantage that if it is extended by supposing that each transverse section of the organ contains many parallel orthotropic 'elements' as in Fig. 3, then it can be applied not only to torsions, but also to plagiotropism, and to the curvatures of the laminae of leaves described by Raydt (1925), if the laminae are supposed to contain similar elements. But it is rather opposed to the observations of the last section on the leaf in the constrained position, since in most parts the cortex of the lower side alone showed growth changes—unless indeed these changes had really nothing to do with the strophic stimulation.

However, Rawitscher, who calls this first hypothesis inadequate, does not consider extending it in the manner of Fig. 3. Instead he suggests (p. 205) that the whole of each transverse section of the main organ is polarized in the dorsiventral direction, in such a way that its dorsal half responds to stimuli like an orthotropic stem, and its ventral half like a root. So his second hypothesis may be derived from the first one by imagining that each of the orthotropic elements shown in a

single row in Fig. 1 is expanded laterally until it fills a whole transverse section. If now the whole organ is placed one on side—say, on the side which is on the right in Fig. 7—then it is to be expected that the lower dorsal quadrant (2 in Fig. 7) and the upper ventral quadrant (3 in Fig. 7) will grow more than the other two, and this may lead to a torsion. Rawitscher supposes indeed that the increase in growth in these two quadrants takes place in *tangential* directions, but it would surely be more consistent with the supposed orthotropic properties of the transverse sections if it took place in directions parallel to their axes of polarity, which stand in the dorsiventral plane of the whole organ: and if it did so, then it would seem more likely to cause a torsion (compare Fig. 2).

Rawitscher also considers that the normal elongation of the whole organ is an essential part of the process and combines somehow with the supposed transverse growth changes in causing the torsion: but to the writer it seems that the normal elongation cannot really make any difference to the tangential forces due to the transverse changes. Of course if the cells are elongating, the tangential forces will divert their elongation into oblique directions. But these forces are surely in principle just as well able to cause a torsion if the cells are not elongating. Unfortunately Rawitscher's second hypothesis is not well supported either by the preliminary observations of the last section. Probably still other forms of 'transverse growth' hypothesis could be suggested.

#### 9. A NOTE ON SOME EXPERIMENTS BY STAUB

Staub (1934) has reported some surprising and puzzling results of experiments concerning torsions, and it seems necessary to consider these briefly, though a thorough review would be too lengthy. Rawitscher (1937) has discussed them briefly already. Staub states that stalks of the objects tested by her, when cut off without laminae, left free at *both* ends and laid on one side or reversed on a glass plate, *rotate* by the shorter way round until the dorsal side is on top. Then they usually stop (p. 211), but sometimes they pass this position and go on rotating until they have transported themselves laterally for distances up to five diameters (p. 235). They do not make *torsions* when free to rotate, but only when fixed at the base; and Staub therefore concludes that the tendency to rotate is primary, and that torsions result merely from the resistance to rotation which is caused by an attachment at one end. The stalks rotate even if they are not supplied with water, and even if they are not growing, or not appreciably, and sometimes even if they are deprived of cortex in one zone: but they do not rotate or make torsions unless fairly mature. Some other surprising results are reported also (pp. 221, 223). By way of contrast it may be mentioned that the ash leaves tested by the writer respond by twisting even when very young (leaves of weeping ash begin to twist when 1.5 or 2 cm. long), that they twist only while still in the growing stage, and that they do not twist if the leaflets are removed, though they may still grow a little.

The objects tested by Staub were hypocotyls of sunflower seedlings, leaves and occasionally flowers of *Viola canadensis* and *V. odorata*, and less frequently flower buds of species of poppy. The first of these objects was strangely chosen. For sunflower hypocotyls are *orthotropic*, and not in any obvious way dorsiventral,

whereas only plagiotropic and dorsiventral organs carry out orientation torsions or 'strophisms'. However Staub claims that the hypocotyls were physiologically dorsiventral as a result of curvatures, which were either the original plumular hooks or more often curvatures previously induced by tropic stimulation; and she states that the convex side was physiologically dorsal. But if so, then during the experiment gravity must have been inducing a new dorsal side, at least in the rooted hypocotyls with which she also worked (for these must have made a second curve), and the conditions must have been very complicated.

The violet leaf stalks were of course plagiotropic and dorsiventral, but in these Staub sometimes made the ventral surface convex by a previous tropic stimulation (p. 215), and the stalks then rotated as if the ventral surfaces were now physiologically dorsal. But in nature the leaf stalks of violets and other plants are sometimes curved with their ventral surfaces convex, and yet they do not twist so as to turn themselves and their laminae upside down! This result therefore suggests the question whether Staub's results, if they can be confirmed, have really anything to do with the strophisms.

Another puzzling point is that it is very difficult to balance curved hypocotyls and stalks on a flat surface with their convex surfaces uppermost, if one tries to do so with one's fingers. Yet we are told (p. 211) that most of them did stop in this position after rotating, and nothing is said to explain how this was possible.

Staub seeks to explain the rotations as due to some hygroscopic mechanism in the stele (pp. 225 seq.), and also (or alternatively?) as due to a shift of the centre of gravity (p. 235). The difficulty for the first suggestion, as she herself recognizes (p. 228), is that hygroscopic mechanisms cause torsions only in one direction, which is structurally determined, whereas the stalks are said to rotate in either direction (p. 206). The second suggestion is difficult to reconcile with the conclusion that rotation is the cause of torsion in attached organs, since the force would be small.

The writer tried to confirm the rotations reported by Staub, and completely failed to do so, but he admits that he did not persist in the attempt as long as he should have done. He suggests that the following two questions need to be answered as a preliminary step concerning her results—first can they be confirmed, and secondly, if so, have the rotations anything to do with the strophisms, or are they due to some quite different process which takes place in relation to a previous curvature, rather than to a plane of dorsiventrality?

#### SUMMARY

1. According to one group of hypotheses the primary growth responses to those stimuli of light or gravity which lead to torsions in dorsiventral organs are changes of some kind in the rates of growth in planes transverse to the long axis of the organ, different changes taking place in different parts of each transverse plane. These changes are supposed to be due to the *orthotropism* of some transverse "elements". According to another hypothesis the primary growth response is that the cells of the organ elongate obliquely in directions depending on the direction of the stimulus. These hypotheses and their consequences are set out and briefly discussed in §§ 1, 2 and 8.



2. If growing pinnate leaves of various woody species are cut off, rotated through  $90^\circ$ , and attached near the tip instead of at the base, the rachis below (or 'proximally' to) the attachment twists in the normal direction in response to gravity, just as when the leaf is attached at the base, bringing its correct face uppermost by the shorter way round.

3. If, however, leaves of *Phaseolus* or leaflets of *Wistaria*, which orientate themselves by pulvini, are cut off, rotated through  $90^\circ$ , and fixed just above (or 'distally' to) the active pulvinus instead of below it, the pulvinus twists in the opposite direction to the normal.

4. It is pointed out that the results with the growing pinnate leaves exclude for them the hypothesis that oblique elongation is primary, and favour the 'transverse growth' hypothesis, while the results with the pulvini exclude for them the latter hypothesis and favour the former.

5. Some anatomical changes are described that took place in the rachis of a young pinnate leaf which was stimulated by gravity for 17 days while prevented mechanically from twisting.

6. Some experiments by Staub (1934) are discussed in § 9.

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## THE DETERMINATION OF AXILLARY BUDS

BY MARY SNOW AND R. SNOW

(With 10 figures in the text)

THE method of operating upon stem apices and leaf primordia which we have practised for other purposes (1931, 1935) makes it possible to investigate what are the causes working in ontogeny which bring it about that lateral buds originate so generally in the axils of leaves, although there are some exceptions. As a preliminary it may be helpful to mention some of the possible explanations which need to be considered or tested. First it may be thought that there is simply nowhere else for them to originate, since in the terminal bud the bases of the leaf primordia are closely packed together. But this does not explain how it is that the buds are so commonly formed only above the morphological centre of the base of the leaf primordium, and not above its marginal parts, where the packing is usually less close. Again, it may be suggested that the positions of leaf and bud are determined by two independent processes. But this can hardly be so, since when leaves are caused to arise in quite abnormal positions, as for instance after our previous operations on *Epilobium hirsutum* (1935), they still have buds in their axils: and it can hardly be supposed that two independent processes are influenced in just the same way by the operations. The positions therefore of leaf and bud must be somehow causally connected. This being so, the possibilities are that either the leaf determines the position of the axillary bud, or that the positions of both are determined by some third common factor: for it will hardly be suggested that the axillary bud determines the position of the subtending leaf, which in the vegetative phase arises as a rule considerably before it.

For the purpose of deciding whether a leaf determines its axillary bud, the most obvious experiment is to cut off a leaf primordium at the youngest stage, before its bud arises, and this operation we have performed on leaf primordia of *Epilobium hirsutum*. In this species in the vegetative phase the youngest three pairs of opposite leaf primordia have regularly no visible axillary buds, but the older pairs all have them. We have published drawings of the normal apices previously (1935, p. 66), and have also described the method of operating (1931, 1935, 1937). Many of the present operations were done at a magnification of 52 times, instead of 30 times.

In the experiments, after removing or bending back those leaves of the terminal bud which arch over the apex and conceal it, we cut off one leaf primordium of the youngest pair that were visible at the time of operation (the  $P_1$ 's, as we have called them) by a cut at its base flush with the surface of the apex or nearly so. About a fortnight later the terminal buds were fixed in alcohol and embedded in collodion; and on examining them by transverse sections, we found that a bud had often been formed in the axil of the removed primordium even when there

remained only an extremely small stump of its base—only enough to appear in two or three free-hand sections. This happened in four out of eight experiments, and the one in which the remains of the base of  $P_1$  were the very smallest is illustrated in Fig. 1. The base of  $P_1$  appeared only in the two sections *a* and *b*.

In the remaining four experiments the axillary bud was not formed, but there was even less trace of the base of the removed primordium, or no trace at all, so that possibly the cut may have injured the presumptive area of the axillary bud,

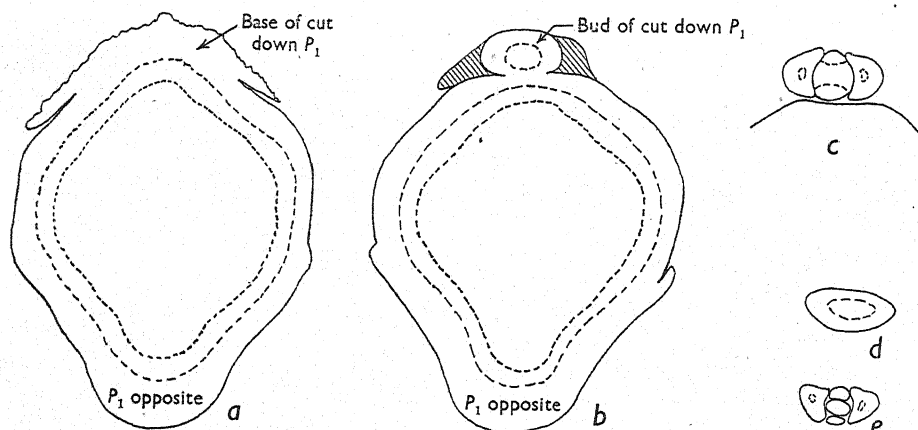


Fig. 1. *Epilobium hirsutum*. A  $P_1$  primordium that was cut down very low, but still formed a bud. *a* and *b* are consecutive sections, lower and higher, at the level of the bases of the  $P_1$ 's. Unorganized tissue is cross-hatched. *c* is the bud of the cut-down  $P_1$ , and *d* and *e* the bud of the intact opposite  $P_1$ , from sections at higher levels. All  $\times 27$ .

that is, the area from which the bud would normally have arisen. In all the above experiments  $P_1$  was large at the time of operation, being near the end of its plastochron.

These results seem to show that the determination of an axillary bud does not need the presence of more than a small basal part, if any, of the upgrowing leaf primordium. But the upgrowing part is probably an outgrowth from a previously determined insertion area or base, and consequently it remains possible that the axillary bud may be determined by the insertion area of the leaf primordium or by a basal zone of its upgrowing part, or by both together. In order to decide whether this is so, it is necessary to destroy the whole leaf primordium without running any risk of injuring the presumptive area of the axillary bud. This cannot be achieved by cutting off the leaf primordium at its base, but we have found that it sometimes results from a cut made in a tangential and nearly vertical plane, passing through the tip of a leaf primordium and down into the axis beneath it (see Fig. 2). This operation, which is difficult, was always performed on a primordium of the youngest pair (a  $P_1$ ) when it was of medium size, being in the middle of its plastochron. The cut either was vertical,

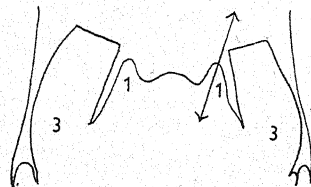


Fig. 2. Diagram of a longitudinal section of a decussate apex, showing the position of a tangential split, marked with a double arrow, through a  $P_1$  of medium size. The  $P_3$ 's are shown cut down.

or more often sloped a little inwards as it descended, and it was seen with certainty at the time of operation that the presumptive area of the bud was not touched.

For this operation three species of Labiatae with very similar apices were used—*Stachys tuberifera*, grown from tubers, and *Stachys silvatica* and *Salvia coccinea*, grown from seed. The last of these species was found the most convenient. The initials of their names will be used as abbreviations. In these species the axillary buds normally become visible first in the axils of the third or fourth pair of opposite leaf primordia.

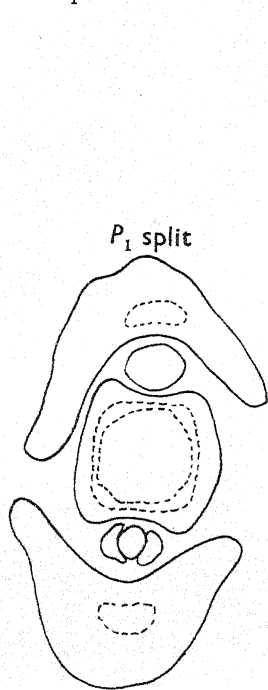


Fig. 3

Fig. 3. *Stachys silvatica*. A tangentially split  $P_1$  which developed as a leaf and had a bud. The opposite  $P_1$  is intact.

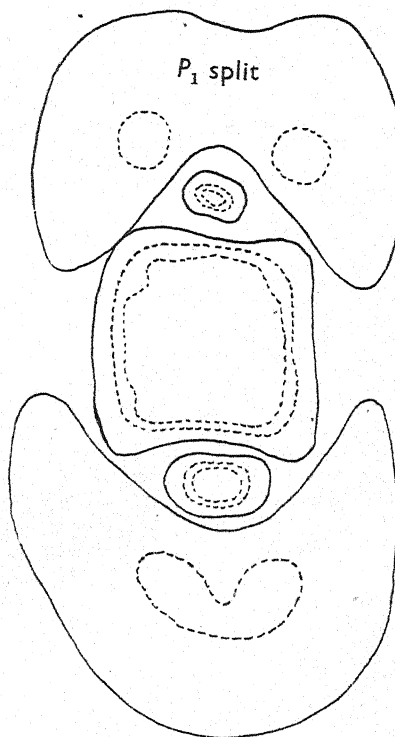


Fig. 4

Fig. 4. *Stachys tuberifera*. Another example of the same.  $\times 31$ .

No difference was noticed between the reactions of the three species, but the development (if any) of the tangentially split  $P_1$  primordium varied greatly, and this was probably because the exact position of the cut varied slightly. On examining by transverse sections the specimens, which in warm weather were fixed and embedded after 2 or 3 weeks, we found that the part of the primordium on the outer side of the cut never developed at all, but in two apices (one *Stachys tuberifera* and one *Salvia coccinea*) a leaf which was rather thin but otherwise nearly normal developed from the part on the inner side of the cut, and had a normal axillary bud. These two are shown in Figs. 3 and 4.

In four other apices there appeared only the scanty remains of the extreme base of a leaf, which must have begun to develop from the inner part of the split



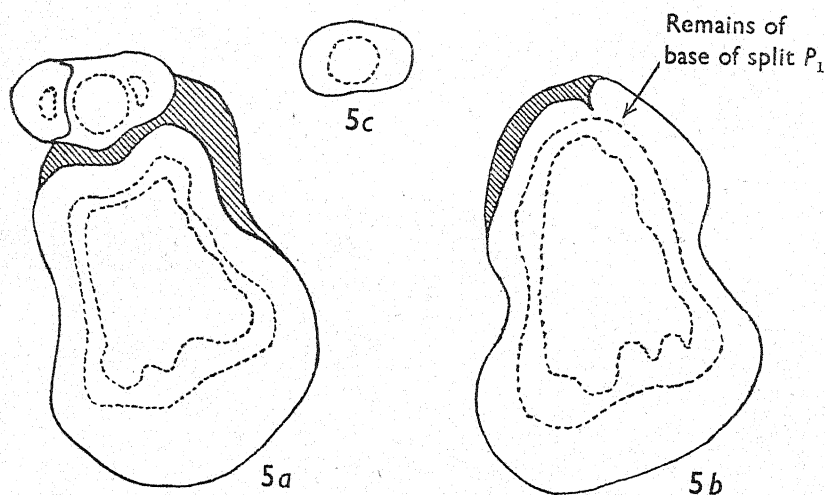


Fig. 5 *a, b, c. Salvia coccinea*. A tangentially split  $P_1$  which had a large bud, though there remained only a small trace of its base. *a* and *b* are sections from slightly different levels, higher and lower. *c* is a section showing the bud of the opposite  $P_1$ . Since the operation the opposite  $P_1$  and its bud had come to be inserted at a higher level than the  $P_1$  operated upon as often happened. The shaded area represents unorganized or moribund tissue. All  $\times 31$ .

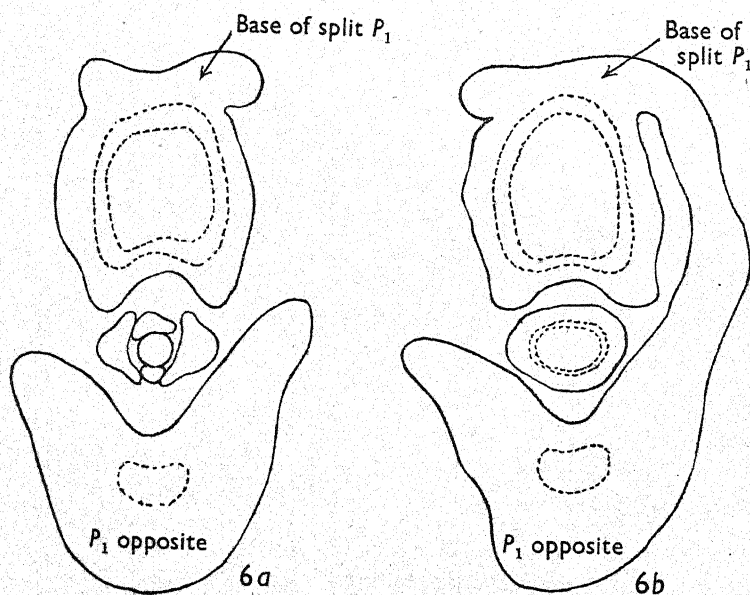


Fig. 6 *a, b. Stachys silvatica*. A tangentially split  $P_1$  which had no bud, and of which there remained only a small trace at the base. *a* and *b* sections from slightly different levels. In *b* the base of the split  $P_1$  is united with the opposite  $P_1$ . Both  $\times 31$ .

primordium and must then have failed. In two of these apices (one *Stachys tuberifera* and one *Salvia coccinea*) these remains had buds in their axils, and in two others (two *Stachys silvatica*) they did not. Examples with and without buds are shown in Figs. 5 and 6. Finally, in four more apices (three *Stachys silvatica* and one *Stachys tuberifera*) there was no trace of the split leaf primordium, which had disappeared completely, and no trace of any axillary bud belonging to it either.

These results show a very clear correlation between development of leaf and presence of axillary bud. They also agree with the previous results in showing that an axillary bud may sometimes be determined even when there remains only a small part of the base of that leaf, together with its insertion area. But they show further that this amount of the leaf is needed, since it is certain that in this series of experiments the presumptive area of the axillary bud was not touched or injured, not even in the four experiments in which there was no trace of the leaf nor of its axillary bud. These results also show that in these species an axillary bud is not yet determined at the middle of the first plastochron of its subtending leaf.

The same conclusions follow from another easier series of experiments in which a  $P_1$  primordium was split vertically in a median *radial* plane, care being taken that the point of the knife should not reach inwards so far as the inner surface of the primordium at its base or the presumptive area of the axillary bud. In four apices thus operated upon (three *Stachys tuberifera* and one *Stachys silvatica*) the split primordium developed almost normally and had a normal axillary bud, the cut having apparently healed up almost completely. In another (*Stachys tuberifera*), which we cannot explain but must consider as exceptional, the split primordium developed normally but had no bud. In two others (one *Stachys tuberifera* and one *Stachys silvatica*), of which one is shown in Fig. 7, only the extreme base of a leaf was found, but it had an axillary bud. Finally, in two more (two *Stachys silvatica*) there was no trace of the split primordium nor of an axillary bud belonging to it.

Although the radial split is easier than the tangential split, it has the disadvantage that one cannot easily be quite so certain that the presumptive area of the bud has not been touched by the knife. The radial splits were made on  $P_1$  primordia which were of small or medium size at the time of operation except in one of the four apices of the first group, in which  $P_1$  was large.

The above results agree in showing that the position of an axillary bud is somehow determined by its subtending leaf, but they seem also to show that for the determination of the bud only a small basal part of the leaf is needed, together with its insertion area. However, the necessary amount of the basal part of the leaf may be more than at first appears. For in those experiments in which only

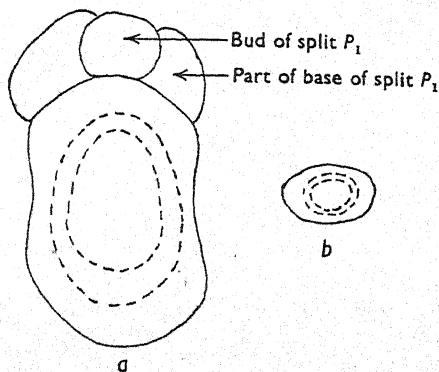


Fig. 7 *a, b. Stachys silvatica*. *a* shows a radially split  $P_1$  which had a bud though there remained only a small part of its base. *b* is the bud of the opposite  $P_1$  from a section at higher level, at same scale.

a very small basal part of a leaf was found, and yet its axillary bud had developed, it is probable that the leaf primordium which was operated upon continued to develop for a little, and then failed and gradually dedifferentiated or died away down to its base. But the bud was determined considerably before the time when the specimens were fixed for examination, though after the operation. So at the time when the bud was determined, a larger proportional amount of its subtending leaf may have been surviving.

Some further evidence concerning bud determination is provided by an earlier series of experiments. After the tangential splits through the  $P_1$  primordia, the part of the primordium on the outer side of the split never developed. But if a rather similar cut is made not through a primordium, but just to the inner side of it, then the primordium, which is now on the outer side of the cut, often develops, and it is of interest to see where, if anywhere, its bud arises. We have made operations of this kind on *Epilobium hirsutum*. The cuts were made just to the inner side of a  $P_1$  primordium, as shown in Fig. 8, and were vertical instead of sloping slightly inwards. They went down to a level below the base of the primordium, which remained connected with the axis below. They were similar to operations which we have performed previously on *Lupinus albus* (1931), and they will be called 'isolations' of primordia as before, although they only partially isolated the primordia from the stem apex. The exact position of the cut was varied purposely, being sometimes close up against the inner surface of the central part of the primordium and sometimes a little way farther in towards the centre of the stem apex.

On fixing the specimens about a fortnight later and on examining them by transverse sections, we found that when a  $P_1$  primordium was isolated and when the cut was some little way away from the inner surface of  $P_1$ , so that the isolated piece was fairly large, then the  $P_1$  always (in seven apices) developed into a nearly complete leaf, and usually (in six of the seven) had a bud; and this bud was always connected directly with it and on the outer side of the cut. But when the cut was made close up against the inner surface of  $P_1$ , then its bud was not formed at all, and the  $P_1$  primordium itself was much reduced, developing either, as in one apex, into a small leaf lacking nearly all its marginal parts, or, as in nine others, only into a small and radially almost symmetric organ, or, as in one more, not developing at all. Thus the bud was never formed on the inner side of the isolating cut, on the stem apex or stem, but only in direct connexion with the leaf. Examples without and with buds are illustrated in Figs. 9 and 10.

These results agree well with the conclusion that an axillary bud is determined by its leaf, since they show that in *Epilobium hirsutum* after an isolation of  $P_1$  a bud is often formed on the isolated leaf, but never on the apex or axis. However, before they can be accepted as further evidence for this conclusion, it must be considered whether they can be explained in another way. For it may be suggested that the bud is determined by some other process, and that the isolating cut sometimes left its presumptive area on the isolated piece, and sometimes passed through that area and destroyed it, but never passed to the outer side of it, and so never left it on the apex. But we think it very unlikely that this was so, since  $P_1$  as it grows gradually extends inwards, spreading farther over the stem apex. Conse-

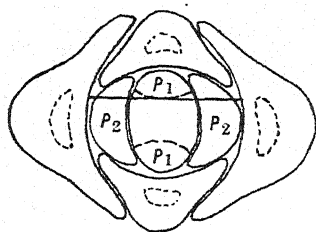


Fig. 8. Normal apex of *Epilobium hirsutum*, in transverse section. The approximate positions of a cut isolating a  $P_1$  is shown by the straight line.  $\times 90$  approx.

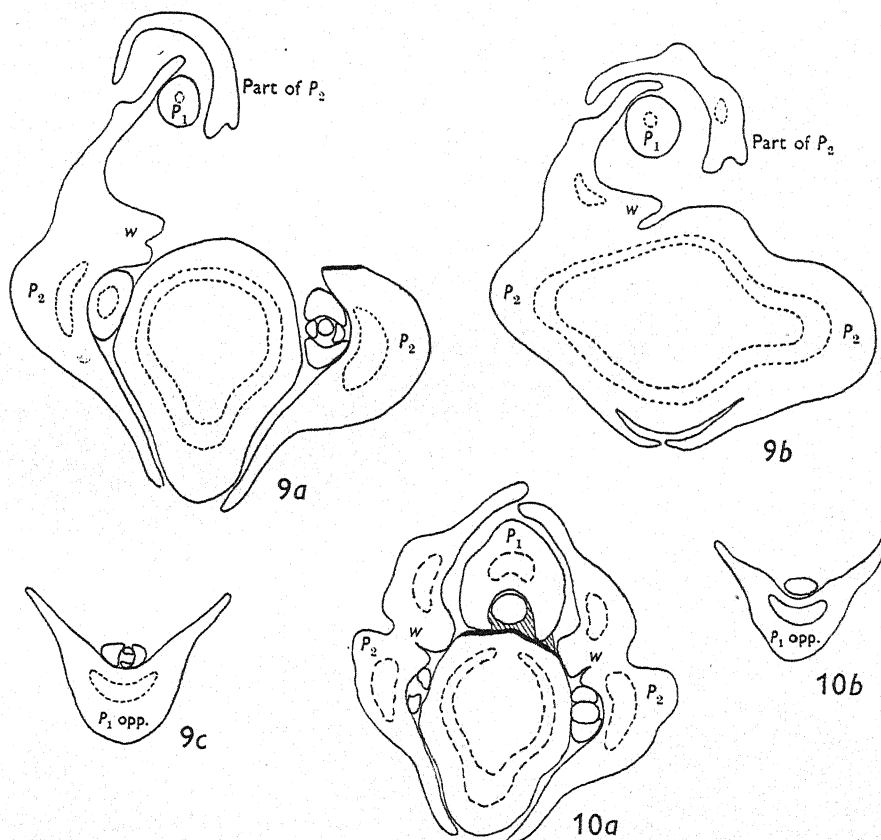


Fig. 9 *a, b, c.* *Epilobium hirsutum*. An isolated  $P_1$  which had no bud and was radial. *a* and *b* are higher and lower sections at a level near base of isolated  $P_1$  showing traces of the cut on the  $P_2$ 's marked *w*. *c* is a section from a higher level through the base of the opposite  $P_1$  with its bud. All  $\times 37$ .

Fig. 10 *a, b.* *Epilobium hirsutum*. An isolated  $P_1$  which had a bud and developed as a leaf. *a* is a section at level of base of isolated  $P_1$  with its bud. A wound scar on the axis is shown in thick black, and traces of the cut on the  $P_2$ 's are marked *w*. Unorganized tissue is cross-hatched. *b* is a section from a higher level showing the base of the opposite  $P_1$  and its bud. Both  $\times 27$ .



quently an isolating cut which was made close up against the inner surface of  $P_1$  when  $P_1$  was small or medium in size (that is, at the beginning or middle of its plastochron) must surely have passed to the outer side of the area from which the axillary bud would normally have been determined at a later time: and actually  $P_1$  was small at the time of operation in nine of the eleven experiments in which no bud was formed, though only in two of the six in which a bud was formed on the isolated leaf. We therefore think it almost certain that in some at least of the eleven experiments in which no bud was formed, the presumptive area of the bud was left on the apex, and that it failed to be determined because it lacked some influence from the leaf.

It is of interest that a bud can be determined on an isolated leaf primordium, since this indicates that the bud-determining influence of the leaf is not a mechanical effect of the axil formed between leaf and stem, but is physiological. For even if a little stem tissue was sometimes included with the isolated piece, the axil must surely soon have been obliterated by the subsequent growth, and no trace of an axil was found at the time of examination.

In *Lupinus albus* also we found (1931, p. 26) that when  $P_1$  was isolated a bud sometimes developed on the isolated leaf, but never on the axis, and the same was true after the isolations of  $I_1$ , that is, of the part of the stem apex from which the next leaf primordium was due to arise, and even after the isolations of  $I_2$ : for after three of the isolations of  $I_2$ , a bud developed on the isolated leaf, a fact not previously mentioned. But in seedlings of that species up to the stage at which the shoot apices were fixed for examination the similar leaves which have not been operated upon have no buds in their axils. Consequently in that species the fact that no bud was ever formed on the apex or axis opposite to an isolated leaf is not evidence that some determining influence from the leaf was lacking. But for the same reason the experiments on *Lupinus albus* add the interesting point that in that species the isolation of a leaf primordium not only permits but greatly accelerates the determination of a bud in connexion with it.

It is further of interest that in *Epilobium* after all the isolations of  $P_1$  in which that primordium had a bud (six in number), its bud was larger at its base, and in two of them much larger, than the bud of the opposite  $P_1$ . In the experiment illustrated (Fig. 10), the bud of the isolated  $P_1$  is only slightly the larger. We reported a rather similar result after some of the diagonal splits of stem apices of *Epilobium* (1935, p. 89): for in these when the split was rather far from median, the smaller of the two pieces formed by it did not regenerate completely, but yet the  $P_1$  belonging to it had a much larger axillary bud than the  $P_1$  of the larger piece. As an explanation we suggested that there was a competition between a tendency of the remaining piece of stem apex to regenerate and a bud determining influence coming from  $P_1$ , so that when the piece of apex was too small to regenerate completely, the axillary bud of  $P_1$  was able to occupy parts of that piece which would not otherwise have been available to it, as indeed it did actually appear to have done. In the present experiments the enlarged buds of the isolated  $P_1$ 's might be explained in a rather similar way. For these buds may have encroached upon tissues adjacent to them on the inner side, which, if they had remained as part of the stem apex, would have taken part in forming younger leaf

primordia, but which on the isolated piece were not able to do so. But in view of the fact, already pointed out, that  $P_1$  spreads inwards over the surface of the apex as it develops, it is doubtful whether much or any tissue was left on the isolated piece on the inner side of the normal presumptive area of the bud of  $P_1$ , even when the isolating cut was one of those which were made some little way from the inner surface of  $P_1$ .

Another possible explanation is that the buds on isolated leaf primordia are determined and develop *earlier* than normal axillary buds, perhaps because they are released from some retarding effect exerted by the stem apex. This certainly happens very conspicuously in *Lupinus albus*, and it may happen to a less degree in *Epilobium*. The two explanations are indeed not entirely different. For common to both is the suggestion that the operations release the isolated leaf primordia (or the smaller pieces of the unequally split stem apices) from some influence opposed to bud formation which is exerted by a stem apex. This influence may either be one which retards the determination and development of axillary buds, as it appears to be in *Lupinus albus*, or one which reduces by competition the area of tissue available for the axillary buds, as we suggested previously for *Epilobium*.

In the experiments on *Epilobium* in which  $P_1$  was cut off at the base and in the tangential and radial splits of  $P_1$  in the Labiatae, the bud of the  $P_1$  operated upon, if it was formed at all, was regularly of about the same size as the bud of the opposite  $P_1$ , even when only a very small basal part of its subtending leaf was found remaining. It seems therefore that the determination of an axillary bud is rather an 'all or nothing' process, and that if a bud is determined at all, it is regularly determined over at least the normal area (or thereabouts) and not later than usual. Only in one of these experiments was the bud of the  $P_1$  operated upon much smaller than the opposite bud (Fig. 4), and this experiment was not one of those in which only a small basal part of  $P_1$  remained, but one in which (after a tangential split)  $P_1$  developed fully, though it was rather thin radially and had its central vascular strand replaced by two lateral ones. This last fact may be somehow connected with the small size of its bud.

In another experiment (Fig. 5) the bud of the tangentially split  $P_1$  was rather larger than that of the opposite  $P_1$ , although there remained only a very small trace of the extreme base of the split  $P_1$ . Perhaps the base of this bud had occupied tissues which had first taken part in the development of the split  $P_1$ , and had then dedifferentiated when  $P_1$  failed.

It should perhaps be mentioned that in some species buds may sometimes be formed in the axils of quite old leaves. But it is probable that these originate from an axillary meristem which is formed much earlier and remains available for bud formation (compare Sandt (1925, p. 59) on the successive development of serial buds from one axillary meristem). The question of distinguishing between the factors determining an axillary meristem and those determining buds within it needs to be further studied.

There is also the question of the determination of such axillary buds as arise, chiefly in inflorescences, before their subtending leaves or bracts. It can hardly be supposed that these are determined by their subtending leaves, and there must therefore presumably be some different process at work.

Again, in some species the factors determining axillary buds must be more complicated. For buds may be formed only in certain of the axils, arranged according to rule, or buds of different kinds may be formed in the same axil. So the attempt at analysis here reported is only a beginning, but we hope that it may point the way for further attempts. We hope to discuss on another occasion the effects of the operations here reported on the subsequent phyllotaxis.

#### SUMMARY

1. The results of various operations on one of the youngest visible pair of leaf primordia in *Epilobium hirsutum* and in various Labiatae indicate that in these species the determination of an axillary bud depends on some influence exerted by the subtending leaf, or by some part of it: the basal part of the leaf, together with its insertion area, is often enough.

2. When in *Epilobium hirsutum* one of the youngest visible pair of primordia is partially isolated from the stem apex by a vertical cut, its bud is always formed on the isolated piece, if it is formed at all, and is abnormally large. This point and others are briefly discussed.

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CROSSING-OVER IN *NEUROSPORA*

By H. L. K. WHITEHOUSE

*Botany School, Cambridge*

(With 6 figures in the text)

## I. GENERAL ACCOUNT

## INTRODUCTION

**N**EUROSPORA is a genus of Ascomycetes belonging to the group Pyrenomycetes. Until Shear & Dodge (1927) described perithecia, the species had been known only by their conidia, which are light and powdery, orange in colour and produced in great abundance. Shear & Dodge found that the species *N. sitophila* and *N. crassa* are heterothallic, existing in two strains, + and -, which are morphologically indistinguishable, perithecia being formed only when + and - come in contact; the asci contain eight spores, black when ripe, arranged in a single row, and in each ascus four of the spores were found to give + mycelia on germination and four to give - mycelia. A third species, *N. tetrasperma*, was found to be homothallic normally, the asci usually containing four spores in each of which a + and a - nucleus is included at its formation (Dodge, 1927).

Wilcox (1928) studied *N. sitophila* cytologically and showed that a single nucleus in the young ascus divides three times in succession and a spore is laid down round each of the eight nuclei so formed. As always in long narrow asci, the spindle of the first division is arranged longitudinally in the ascus. The two spindles of the second division are also longitudinal, and they are well apart so that the two centre nuclei do not pass. The four spindles of the third division are transverse and some distance apart. Wilcox was the first to isolate the spores in order from individual asci, and the work of Lindegren (1933) has confirmed that in both *N. sitophila* and *N. crassa*, numbering from the top end of the ascus, spores 1 and 2, 3 and 4, 5 and 6, 7 and 8, i.e. adjacent pairs of spores, are always identical (see Fig. 1). Clearly the third nuclear division in the ascus is a simple mitosis. In asci showing segregation for a single pair of allelomorphic genes (e.g. the sex genes, + and -), there are six possible arrangements of the spores, and these arrangements can be grouped into two classes (see Table 1); either the two pairs of spores in one half of the ascus are both of one sex, the two pairs in the other half being of the other sex (class 1), or each half of the ascus contains a pair of each sex (class 2). Class 1 asci are the result of segregation occurring at the first nuclear division in the ascus, whilst class 2 asci result from second division segregation (Fig. 1). Evidently the first two divisions in the ascus constitute meiosis.

In *N. tetrasperma*, Dodge (1927) and Colson (1934) have shown that the two spindles at the second division elongate greatly so that the two centre nuclei pass each other. The possibility that such nuclear passing occurs occasionally in *N. sitophila* and *N. crassa* must not be overlooked, and its effects are considered on p. 38 et seq.



The value of *N. sitophila* and *N. crassa* for genetical work lies in the following characteristics:

- (1) They are heterothallic, enabling mutant strains to be crossed with ease.
- (2) All four products of a single meiosis can be isolated and grown to maturity; moreover, as in no other organism, it is possible to determine from the order of the four pairs of spores at which of the divisions segregation occurred.
- (3) *Neurospora* grows very readily in culture, and the heat necessary to germinate the ascospores at the same time kills the conidia.

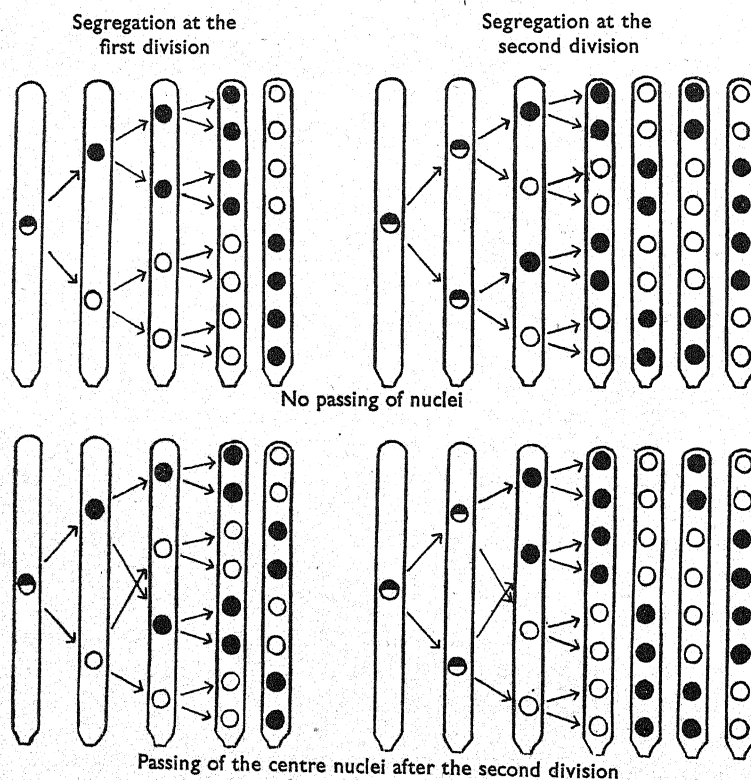


Fig. 1. Diagrams of the spore arrangements in asci of *Neurospora sitophila* and *N. crassa* that would result from first and from second division segregation for a pair of allelomorphs, with and without passing of the centre nuclei after the second division.

Table 1

Pairs of spores				Class	Simplest cross-overs
1	2	3	4		
+	+	-	-	1	Non-cross-over.
-	-	+	+		
+	-	+	-	2	1 cross-over
+	+	-	+		
-	-	+	-		

(4) The sexual life cycle is completed in two or three weeks, so the generation time is short.

(5) Meiosis follows directly upon nuclear fusion, so that the fungus plant is haploid and there are none of the complications associated with dominance and recessiveness found in diploid organisms. The mycelium, however, may be heterokaryotic, i.e. contain nuclei of more than one kind, and then it is quite possible for the effect of a gene in one kind of nucleus to be masked by a gene in another. Cultures derived from single ascospores, however, can only become heterokaryotic by mutation.

*N. tetrasperma*, being usually homothallic, is much less suitable than the heterothallic species for genetical study. This paper is concerned solely with *N. sitophila* and *N. crassa*.

#### CROSSING-OVER AND SEGREGATION

During meiosis the centromeres (or spindle attachments) of the paired chromosomes separate undivided at anaphase of the first division. Hence if the locus of a gene is very close to the centromere of its chromosome it will always segregate at the first division. If a cross-over occurs proximally to the locus, i.e. between it and the centromere, segregation will not occur until the second division of meiosis (see Fig. 2). Hence for any given gene, the proportions of first and second division

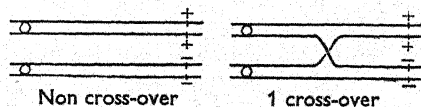


Fig. 2. Diagrammatic representations of the sex chromosomes at diplotene of meiosis, with no crossing-over and with a single cross-over proximal to the sex gene.

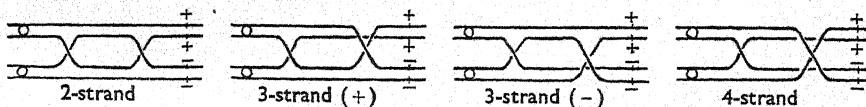


Fig. 3. Diagrammatic representations of the sex chromosomes at diplotene of meiosis to show the four different relationships of chromatids possible between two cross-overs.

segregation will be constant, and the percentage of second division segregation is the percentage of asci (i.e. of meioses) in which a cross-over occurs proximal to the gene. Now if a cross-over occurs in a region between two genes, the four products of meiosis will consist of two original combinations of the genes and two recombinations, since crossing-over occurs at the four-strand stage. In other words, an average of one cross-over in this region per ascus gives 50% recombination between the genes delimiting the region, and the map distance between the genes is 50 units (uncorrected for double cross-overs), since the uncorrected distance between two genes on a genetic map of a chromosome is the percentage of recombination. Now if one cross-over occurs regularly between the locus of a gene and the centromere there will be 100% second division segregation; also, the distance of the gene from the centromere will be 50 units. Thus half the percentage of second division segregation for a gene is the uncorrected map distance of the gene from the centromere.

The effect of two cross-overs proximal to the locus of a gene must now be considered. Double cross-overs may be two-strand (reciprocal), three-strand (diagonal or disparate) or four-strand (complementary) (see Fig. 3). Two-strand and four-strand double cross-overs proximal to a gene will cause first division segregation, whilst three-strand will result in segregation at the second division. Clearly two-strand and four-strand double cross-overs will be indistinguishable from no crossing-over, and three-strand from single cross-overs. Thus with asci showing segregation for only a single pair of allelomorphs double cross-overs are not detectable.

If mutant strains of *Neurospora* are crossed with the wild type or 'normal' strain, then by dissecting individual hybrid asci and isolating the spores in order, it is possible to map the genes in relation to the centromere of the chromosome as well as in relation to one another: half the percentage of second division segregation gives the distance from the centromere, and the percentage of recombination gives the distance between gene loci. With a set of linked genes it is possible to determine the cross-overs that took place at meiosis, from the spore arrangements in asci showing segregation for all the genes. In particular it is possible in many cases to distinguish two-strand, three-strand and four-strand double cross-overs, and hence to test for chromatid interference. On a hypothesis of random crossing-over the expected proportion of two-strand : three-strand : four-strand double cross-overs is 1 : 2 : 1. A significant deviation from this proportion would mean there was chromatid interference in crossing-over. Chiasma interference can also be detected from the frequencies of the different spore arrangements.

#### ASCOSPORE ARRANGEMENTS

With asci showing segregation for a single pair of allelomorphs, there are six possible arrangements of the spores in the ascus, as described above, and they can be grouped into two classes (Table 1). With two pairs of allelomorphs there are thirty-six possible arrangements of the spores in the ascus. If the four pairs of spores in order from the top end of the ascus are denoted by the letters  $ABCD$ , it is clear that the transposition of  $A$  and  $B$  and of  $C$  and  $D$  to give  $BACD$ ,  $ABDC$  and  $BADC$  could be brought about in the ascus simply by reversing the spindles at the second division; similarly  $CDA B$ ,  $CDBA$ ,  $DCAB$  and  $DCBA$  would be the corresponding spore arrangements had the first division spindle been reversed. These eight arrangements will occur with statistically equal frequencies and can be grouped together. The thirty-six spore arrangements possible in asci showing segregation for two pairs of allelomorphous genes can be grouped in this way into seven classes. This is shown in Table 2.  $X$  and  $Y$  are the mutant forms and  $x$  and  $y$  their respective normal allelomorphs.<sup>1</sup> The six possible arrangements for  $X$  and  $x$  are shown down the left-hand side, and the six for  $Y$  and  $y$  across the top, the thirty-six possibilities being given by the intersections of columns and rows. The numbers from 1 to 7 indicate the seven classes. In the case

<sup>1</sup> The large and small letters are used to indicate that the genes form an allelomorphous pair; there is no question of  $X$  being dominant over  $x$  (or  $Y$  over  $y$ ), since it is only in the single diploid nucleus in the young ascus that both are present in the same nucleus.

of classes 1 and 2 there are only two arrangements within each; in classes 5 and 6 there are four arrangements, whilst in classes 3, 4 and 7 there is the maximum of eight arrangements.

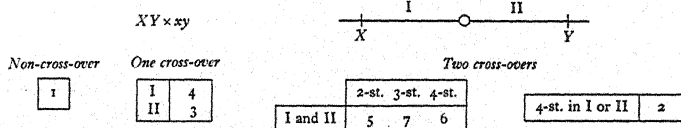
Asci showing segregation for three pairs of allelomorphs will give  $6^3 = 216$  possible spore arrangements. Grouping together, as before, arrangements which differ merely in the spindle orientations at the first and second divisions, there are thirty-two classes of arrangements (Table 3). The three genes are denoted by  $X$ ,  $Y$  and  $Z$  and their normal allelomorphs by  $x$ ,  $y$  and  $z$ . Only two of the six possible arrangements of  $X$  and  $x$  are shown ( $XXxx$  and  $XxXx$ ), because the classes of

Table 2. *Two gene loci*

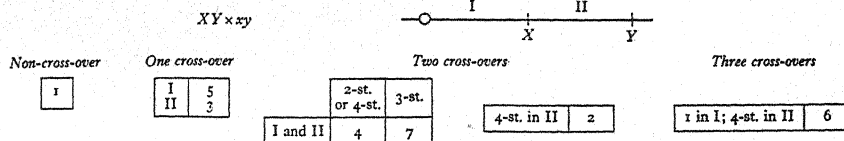
The thirty-six possible arrangements of the spores in asci showing segregation for two pairs of allelomorphs can be grouped into seven classes:

Pairs of spores	1	2	3	4	5	6	7
1	$Y$	$y$	$Y$	$y$	$Y$	$y$	$Y$
2	$y$	$Y$	$y$	$Y$	$y$	$Y$	$y$
3	$y$	$Y$	$Y$	$y$	$y$	$Y$	$Y$
4	$Y$	$y$	$Y$	$Y$	$y$	$y$	$y$
5	$X$	$x$	$x$	$x$	$1$	$2$	$3$
6	$x$	$X$	$X$	$X$	$2$	$1$	
7	$X$	$x$	$X$	$x$	$5$	$6$	$7$
8	$x$	$X$	$x$	$X$	$6$	$5$	
9	$X$	$x$	$x$	$X$	$7$	$6$	$5$
10	$x$	$X$	$X$	$x$			

Two gene loci, one in each arm



Two gene loci in one arm



spore arrangements given by the other four are readily deduced from these two.

For instance, arrangement  $xXXx$  gives  $YyyY$  on reversing the first two pairs of spores, and from the table this belongs in class 30. Thus before using Table 3,  $X$  and  $x$  must be put in the form  $XXxx$  or  $XxXx$ , either by reversing the order as a whole, or by reversing the two pairs of spores in the first half of the ascus (as in the example above) or the two in the second half.

With four pairs of allelomorphs, there are  $6^4 = 1296$  arrangements of the spores, and these can be grouped into 172 classes (Table 4a). The four genes are denoted by  $W$ ,  $X$ ,  $Y$  and  $Z$  and their normal allelomorphs by  $w$ ,  $x$ ,  $y$  and  $z$ . Only seven of the thirty-six possible combined arrangements for  $W$  and  $X$  are shown, corresponding to the seven classes in Table 2. Hence before using Table 4(a) to find the class to which a particular spore arrangement belongs,  $W$  and  $X$  must be put





into one of the seven arrangements shown, either by reversing the order as a whole, or by reversing the two pairs of spores in the first half of the ascus, or the two in the

second half, or both. For example, arrangement  $\begin{matrix} w & W & w & W \\ X & X & x & x \\ Y & y & y & Y \\ z & Z & Z & z \end{matrix}$  gives  $\begin{matrix} W & w & W & w \\ X & X & x & x \\ y & Y & Y & y \\ Z & z & z & Z \end{matrix}$  on

reversing both halves of the ascus separately and from the table this belongs in class 95.

Table 4a. Four gene loci

The 1296 possible arrangements of the spores in asci showing segregation for four pairs of allelomorphs can be grouped into 172 classes:

$WWww$ $XXxx$											
Pairs of spores	1	2	3	4	5	6	7	8	9	10	11
1	2	3	4	5	6	7	8	9	10	11	12
Y Y y y	1	2	3	4	5	6	7	8	9	10	11
y y Y Y	12	13	14	15	16	17	18	19	20	21	22
Y y Y y	23	24	25	26	27	28	29	30	31	32	33
y Y y Y	34	35	36	37	38	39	40	41	42	43	44
Y y y Y	45	46	47	48	49	50	51	52	53	54	55
y Y Y y	56	57	58	59	60	61	62	63	64	65	66

$WWww$ $xxXX$											
Pairs of spores	1	2	3	4	5	6	7	8	9	10	11
1	2	3	4	5	6	7	8	9	10	11	12
Y Y y y	13	14	15	16	17	18	19	20	21	22	23
y y Y Y	24	25	26	27	28	29	30	31	32	33	34
Y y Y y	35	36	37	38	39	40	41	42	43	44	45
y Y y Y	46	47	48	49	50	51	52	53	54	55	56
Y y y Y	57	58	59	60	61	62	63	64	65	66	67
y Y Y y	68	69	70	71	72	73	74	75	76	77	78

$WwWw$ $XXxx$											
Pairs of spores	1	2	3	4	5	6	7	8	9	10	11
1	2	3	4	5	6	7	8	9	10	11	12
Y Y y y	61	62	63	64	65	66	67	68	69	70	71
y y Y Y	72	73	74	75	76	77	78	79	80	81	82
Y y Y y	83	84	85	86	87	88	89	90	91	92	93
y Y y Y	94	95	96	97	98	99	100	101	102	103	104
Y y y Y	105	106	107	108	109	110	111	112	113	114	115
y Y Y y	116	117	118	119	120	121	122	123	124	125	126

$WwWw$ $xxXX$											
Pairs of spores	1	2	3	4	5	6	7	8	9	10	11
1	2	3	4	5	6	7	8	9	10	11	12
Y Y y y	117	118	119	120	121	122	123	124	125	126	127
y y Y Y	128	129	130	131	132	133	134	135	136	137	138
Y y Y y	139	140	141	142	143	144	145	146	147	148	149
y Y y Y	150	151	152	153	154	155	156	157	158	159	160
Y y y Y	161	162	163	164	165	166	167	168	169	170	171
y Y Y y	172	173	174	175	176	177	178	179	180	181	182

If asci show segregation for  $x$  pairs of allelomorphs, the number of spore arrangements is  $6^x$  and the number of classes into which these can be grouped is given by the empirical formula

$$\frac{3}{2}(x+1)! - 2^{x-1}.$$

Table 4b. Four gene loci, two in each arm

WXYZ × wxyz

Two cross-overs

Non-  
cross-over

I

One cross-over

I	61
II	97
III	9
IV	5

	2-st.	3-st. (X)	3-st. (x)	4-st.
I and II	25	140	137	28
I and III	77	96	93	80
I and IV	65	70	69	66
II and IV	99	102	101	100
III and IV	7	12	11	8

	2-st.	3-st.	4-st.
II and III	105	115	108

4-st. in I	16
4-st. in II or III	4
4-st. in IV	2

Three cross-overs

	2-st.				3-st. (X)				3-st. (x)				4-st.			
	2-st.	3-st.	3-st. (X)	4-st.	2-st.	3-st.	3-st. (X)	4-st.	2-st.	3-st.	3-st. (X)	4-st.	2-st.	3-st.	3-st. (X)	4-st.
I, II and IV	29	34	33	30	147	143	144	148	145	142	141	146	31	35	36	32
I, III and IV	73	82	81	74	87	91	92	88	85	90	89	86	75	83	84	76

	2-st. or 4-st.			3-st.		
	2-st.	3-st.	4-st.	2-st.	3-st.	4-st.
I, II and III	41	57 and 60	44	169	153 and 156	172

4-st. in I; 1 in II	117
4-st. in I; 1 in III	21
4-st. in I; 1 in IV	18
1 in I; 4-st. in II or III	64

4-st. in II or III; 1 in IV	6
1 in I; 4-st. in IV	62
1 in II; 4-st. in IV	98
1 in III; 4-st. in IV	10

	2-st.		3-st.		4-st.	
	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.
II, III and IV	103	109	111 and 112	113 and 114	104	110

Four cross-overs

	2-st. or 4-st.						3-st.					
	2-st.		3-st.		4-st.		2-st.		3-st.		4-st.	
	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.
I, II, III and IV	37 and 38	45 and 46	49, 50, 51 and 52	53, 54, 55 and 56	39 and 40	47 and 48	161, 162	165 and 166	149, 150, 151 and 152	157, 158, 159 and 160	163 and 164	167 and 168

	2-st.	3-st. (X)	3-st. (x)	4-st.
I and II; 4-st. in IV	26	139	138	27
I and III; 4-st. in IV	78	95	94	79
4-st. in I; II and IV	120	121	122	119
4-st. in I; III and IV	20	23	24	19

	2-st.	3-st.	4-st.
II and III; 4-st. in IV	106	116	107
4-st. in I; II and III	128	135	125

	4-st. in I; 4-st. in II or III	13
4-st. in I; 4-st. in IV	15	
4-st. in II or III; 4-st. in IV	3	

1 in I; 4-st. in II or III; 1 in IV 67, 68, 71 and 72

Five cross-overs

	2-st.		3-st.		4-st.	
	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.
4-st. in I; II, III and IV	124	130	131 and 132	133 and 134	123	129

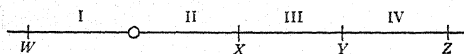
4-st. in I; 4-st. in II or III; 1 in IV	17
4-st. in I; 1 in II; 4-st. in IV	118
4-st. in I; 1 in III; 4-st. in IV	22
1 in I; 4-st. in II or III; 4-st. in IV	63

	2-st. or 4-st.			3-st.		
	2-st.	3-st.	4-st.	2-st.	3-st.	4-st.
I, II and III; 4-st. in IV	42	58 and 59	43	170	154 and 155	171

Six cross-overs

	2-st.	3-st.	4-st.
4-st. in I; II and III; 4-st. in IV	127	136	126

4-st. in I; 4-st. in II or III; 4-st. in IV	14
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$$WXYZ \times wxyz$$


### Two cross-overs

Non-  
cross-over

### One cross-over

I	61
II	41
III	9
IV	5

	2-st.	3-st. ( $\bar{X}$ )	3-st. ( $\bar{x}$ )	4-st.
I and III	77	96	93	80
I and IV	65	70	69	66
II and III	25	60	57	28
II and IV	37	46	45	38
III and IV	7	12	11	8

	2-st.	3-st.	4-st.
I and II	105	169	128

4-st. in I or II	16
4-st. in III	4
4-st. in IV	2

### Three cross-overs

	2-st.				3-st. ( $X$ )				3-st. ( $x$ )				4-st.			
	2-st.	3-st. ( $X$ )	3-st. ( $x$ )	4-st.	2-st.	3-st. ( $X$ )	3-st. ( $x$ )	4-st.	2-st.	3-st. ( $X$ )	3-st. ( $x$ )	4-st.	2-st.	3-st. ( $X$ )	3-st. ( $x$ )	4-st.
I, III and IV	73	82	81	74	87	91	92	88	85	90	89	86	75	83	84	76
II, III and IV	29	34	33	30	55	51	52	56	53	50	49	54	31	35	36	32

	2-st.		3-st.		4-st.	
	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.
I, II and III	97	115	137 and 140	153 and 156	117	135
I, II and IV	103	100	161 and 162	165 and 166	124	130

4-st. in I or II; 1 in III	21
4-st. in I or II; 1 in IV	18
1 in I; 4-st. in III	64
1 in II; 4-st. in III	44

4-st. in III; 1 in IV	6
1 in I; 4-st. in IV	62
1 in II; 4-st. in IV	42
1 in III; 4-st. in IV	10

### Four cross-overs

		FOUR CROSS-OVERS															
I		2-st.					3-st.					4-st.					
II		2-st. or 4-st.		3-st.			2-st. or 4-st.		3-st.			2-st. or 4-st.		3-st.			
III		2-	3-(X)	3-(x)	4-	2-	3-(X)	3-(x)	4-	2-	3-(X)	3-(x)	4-	2-	3-(X)	3-(x)	4-
IV																	
		145	142	141	146	157	150	149	158								
		and	and	and	and	and	and	and	and								
		147	143	144	148	159	151	152	160	120	121	122	119	134	131	132	133

	2-st.	3-st. (X)	3-st. (x)	4-st.
4-st. in I or II; III and IV	20	23	24	19
I and III, 4-st. in IV	78	95	94	79
II and III; 4-st. in IV	26	59	58	27

	2-st.	3-st.	4-st.
I and II; 4-st. in III	108	172	125
I and II; 4-st. in IV	106	170	127

4-st. in I or II; 4-st. in III	13
4-st. in I or II; 4-st. in IV	15
4-st. in III; 4-st. in IV	3

1 in I; 4-st. in III; 1 in IV	67, 68, 71 and 72
1 in II; 4-st. in III; 1 in IV	39, 40, 47 and 48

### Five cross-overs

	2-st.		3-st.		4-st.	
	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.	2-st. or 4-st.	3-st.
I, II and III; 4-st. in IV	98	116	138 and 139	154 and 155	118	136

4-st. in I or II; 4-st. in III; 1 in IV	17
4-st. in I or II; 1 in III; 4-st. in IV	22
1 in I; 4-st. in III; 4-st. in IV	63
1 in II; 4-st. in III; 4-st. in IV	43

	2-st.	3-st.	4-st.
I and II; 4-st. in III; 1 in IV	104 and 110	163, 164, 167 and 168	123 and 129

### Six cross-overs

	2-st.	3-st.	4-st.
I and II; 4-st. in III; 4-st. in IV	107	171	126

4-st. in I or II; 4-st. in III; 4-st. in IV	14
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Table 4d. Four gene loci in one arm

WXYZ × wxyz

Non-cross-over

I

One cross-over

I	105
II	41
III	9
IV	5

	2-st. or 4-st.	3-st.
I and II	61	169
I and III	97	115
I and IV	103	109

Two cross-overs

	2-st.	3-st. (X)	3-st. (x)	4-st.
II and III	25	60	57	28
II and IV	37	46	45	38
III and IV	7	12	11	8

4-st. in II	16
4-st. in III	4
4-st. in IV	2

Three cross-overs

	2-st.	3-st. (X)	3-st. (x)	4-st.												
2-st.	3-st. (X)	3-st. (x)	4-st.	2-st.	3-st. (X)	3-st. (x)	4-st.	2-st.	3-st. (X)	3-st. (x)	4-st.	2-st.	3-st. (X)	3-st. (x)	4-st.	
II, III and IV	29	34	33	30	55	51	52	56	53	50	49	54	31	35	36	32

	2-st. or 4-st.	3-st.						
2-st.	3-st. (X)	3-st. (x)	4-st.	2-st.	3-st. (X)	3-st. (x)	4-st.	
I, II and III	77	96	93	80	153	140	137	156
I, II and IV	65	70	66	66	105	102	101	106
I, III and IV	99	102	101	100	113	112	111	114

I in I; 4-st. in II	128
4-st. in II; I in III	21
4-st. in II; I in IV	18
I in I; 4-st. in III	108
I in II; 4-st. in III	44

4-st. in III; I in IV	6
I in I; 4-st. in IV	106
I in II; 4-st. in IV	42
I in III; 4-st. in IV	10

Four cross-overs

	2-st. or 4-st.	3-st.																													
2-st.	3-st. (X)	3-st. (x)	4-st.	2-st.	3-st. (X)	3-st. (x)	4-st.																								
2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-	2- (X)3-(x)4-																
73	82	81	74	87	91	92	88	85	90	89	86	75	83	84	76	157	150	149	158	147	143	144	148	145	142	141	146	159	151	152	160

4-st. in II; III and IV	20	23	24	19
II and III; 4-st. in IV	26	59	58	27

I in I; 4-st. in III; I in IV	104 and 110
I in II; 4-st. in III; I in IV	39, 40, 47 and 48

	2-st. or 4-st.	3-st.
I and II; 4-st. in III	64	172
I and II; 4-st. in IV	62	170
I and III; 4-st. in IV	98	116

4-st. in II; 4-st. in III	13
4-st. in II; 4-st. in IV	15
4-st. in III; 4-st. in IV	3
I in I; 4-st. in II; I in III	117 and 135
I in I; 4-st. in II; I in IV	124 and 130

Five cross-overs

	2-st. or 4-st.	3-st.						
2-st.	3-st. (X)	3-st. (x)	4-st.	2-st.	3-st. (X)	3-st. (x)	4-st.	
I, II and III; 4-st. in IV	78	95	94	79	154	139	138	155

	2-st.	3-st. (X)	3-st. (x)	4-st.
I in I; 4-st. in II; III and IV	120 and 134	121 and 131	122 and 132	119 and 133

	2-st. or 4-st.	3-st.
I and II; 4-st. in III; I in IV	67, 68, 71 and 72	163, 164, 167 and 168

I in I; 4-st. in II; 4-st. in III	125
I in II; 4-st. in III; I in IV	17
I in I; 4-st. in II; 4-st. in IV	127
4-st. in II; I in III; 4-st. in IV	22
I in I; 4-st. in III; 4-st. in IV	107
I in II; 4-st. in III; 4-st. in IV	43

Six cross-overs

	2-st. or 4-st.	3-st.
I and II; 4-st. in III; 4-st. in IV	63	171

I in I; 4-st. in II; 4-st. in III; I in IV	123 and 129
I in I; 4-st. in II; I in III; 4-st. in IV	118 and 136
4-st. in II; 4-st. in III; 4-st. in IV	14

Seven cross-overs

I in I; 4-st. in II; 4-st. in III; 4-st. in IV	126
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## DETECTABLE CROSS-OVERS

The number of classes of spore arrangements in the asci depends solely on the number of genes for which the asci show segregation. The genes may or may not be on separate chromosomes. When the genes are linked, the spore arrangements enable the cross-overs that occurred at meiosis to be deduced. The cross-overs detectable in this way depend on the disposition of the gene loci in relation to the centromere. In the case of two pairs of allelomorphs there are two possibilities: either the two genes are located on opposite sides of the centromere, or they are both on the same side. The simplest cross-overs that would give the seven classes of spore arrangements are shown in Table 2 for each of these possibilities. It is assumed that the cross is made between  $XY$  and  $xy$ . If the parental strains were  $Xy$  and  $xY$ ,  $Y$  must be interchanged with  $y$  throughout the asci, before reference is made to the table; moreover, with two genes in one arm,  $X$  must be the proximal and  $Y$  the distal gene. In describing the cross-overs a semi-colon placed between two regions (e.g. '1 in I; 4-st. in II') means that the relationship of the adjacent cross-overs may be of any kind (two-strand, three-strand or four-strand).

When the asci show segregation for three pairs of allelomorphs, there are again two possible arrangements for the genes, assuming they are all located in one chromosome; either they may be two on one side of the centromere and one on the other side, or all three on the same side. The simplest cross-overs to give the thirty-two classes of spore arrangements for each of these cases are shown in Table 3. It is assumed that  $XYZ \times xyz$  was the parental cross, and that the dispositions of  $X$ ,  $Y$  and  $Z$  in relation to the centromere and to one another are as shown. Between certain regions the two kinds of three-strand double cross-over (Fig. 3) give different classes of spore arrangement. '3-st. ( $X$ )' refers to the double cross-over in which the third strand, involved in the second cross-over but not in the first, is a strand derived from the  $XYZ$  parent; similarly '3-st. ( $x$ )' means the new strand involved at the second cross-over is from the  $xyz$  parent.

With four-linked genes, there are three possible arrangements of the genes in relation to the centromere: two in each arm, three in one arm and one in the other, or all four in one arm. For each of these arrangements, the simplest cross-overs to give the 172 classes of spore arrangements are shown in Tables 4(b), 4(c) and 4(d) respectively.  $WXYZ$  and  $wxyz$  are the parental types, and the relative positions of  $W$ ,  $X$ ,  $Y$  and  $Z$  in each case are as shown. Some of the cross-overs are rather complex and two examples will be given to show the use of the tables. With four genes, two in each arm, an ascus in class 47 is the result of four cross-overs, one in each of the regions, the relationship between the cross-overs in regions I and II being either two-strand or four-strand, that between II and III being four-strand and that between III and IV being three-strand. With the same arrangement of genes an ascus in class 130 is due to five cross-overs, two in region I and one in each of the other regions, the relationship between the two cross-overs in region I being four-strand, that between the nearest cross-over in region I and the cross-over in region II being of any kind, that between II and III being two-strand, and that between III and IV being three-strand.

In an ascus showing segregation for a number of linked genes, the cross-overs

detectable from the arrangement of the spores are a combination of those detectable from the divisions at which the genes segregate and those detectable by recombination. In a region adjoining the centromere only single cross-overs can be detected; for, as has already been shown, two-strand and four-strand double cross-overs give first division segregation, the same as no crossing-over, and three-strand double cross-overs give second division segregation, the same as single cross-overs (Fig. 3). In a region bounded on both sides by genes, four-strand double cross-overs within the region are detectable as well as single cross-overs; for two-strand double cross-overs give no recombination types amongst the four products of meiosis, the same as no crossing-over; three-strand double cross-overs give two recombination types and two original combination, the same as single cross-overs; and four-strand double cross-overs give all four recombined. Consequently, from the spore arrangements in the asci the following cross-overs can be detected:

- (1) All combinations of 0 or 1 cross-over in each region.
- (2) All combinations of four-strand double cross-overs within regions bounded by genes (but not in regions bounded by a gene and the centromere) and 0 or 1 cross-over in each remaining region.

The following cross-overs cannot be detected:

- (1) Two-strand and three-strand double cross-overs within any region and four-strand double cross-overs within a region bounded at one end by the centromere.
- (2) Three or more cross-overs within a region.

Map distances between adjacent genes obtained from recombination data cannot be corrected by means of the cross-overs deduced from the spore arrangements, except across the centromere, since in all other regions the cross-overs detected by ascospore arrangements are the same as those detected by recombination. In the case of two genes adjoining the centromere one on each side of it, recombination fails to include two-strand double cross-overs across the centromere and includes three-strand double cross-overs across the centromere as single cross-overs. The sum of the two distances obtained by halving the respective percentages of second division segregation, on the other hand, includes these cross-overs, but fails to record four-strand double cross-overs both in the same arm, which were detected by recombination. Thus by adding to the cross-overs detectable by second division segregation, the four-strand double cross-overs both in the same arm detectable by recombination, a better estimate of the map distance between the two genes is given.

With two cross-overs proximal to the locus of a gene, as shown above, two-strand and four-strand relationships will cause first division segregation, whilst three-strand will result in segregation at the second division. Hence, if there is no chromatid interference, two cross-overs will give 50% second division segregation. In the same way it can be shown that three cross-overs will give 75% second division segregation, four will give 62.5% and five will give 68.75%. Mather (1935) has shown that with  $n$  cross-overs, the percentage of second division segregation is

$$66.7 \left[ 1 - \left( -\frac{1}{2} \right)^n \right].$$

The limiting value of this expression as  $n$  tends to  $\infty$  is 66.7%. As the terms of

the series alternate above and below the limiting value and in view of the variability in the number of cross-overs in a given region, percentages of second division segregation greatly in excess of 66.7 are likely to be rare.

# CHROMATID INTERFERENCE

Chromatid interference in crossing-over is shown by a deviation of the proportion of two-strand : three-strand : four-strand double cross-overs from the proportion 1 : 2 : 1 expected on a random hypothesis. The two cross-overs may be in different regions or both in the same region.

## (a) Two cross-overs in different regions

The four possible kinds of double cross-overs (two-strand, three-strand (X), three-strand (x) and four-strand) all give different spore arrangements (type A, Fig. 4), except in the following cases:

(i) Between regions adjoining the centromere on each side of it, the two kinds of three-strand double cross-over are indistinguishable (type B, Fig. 4).

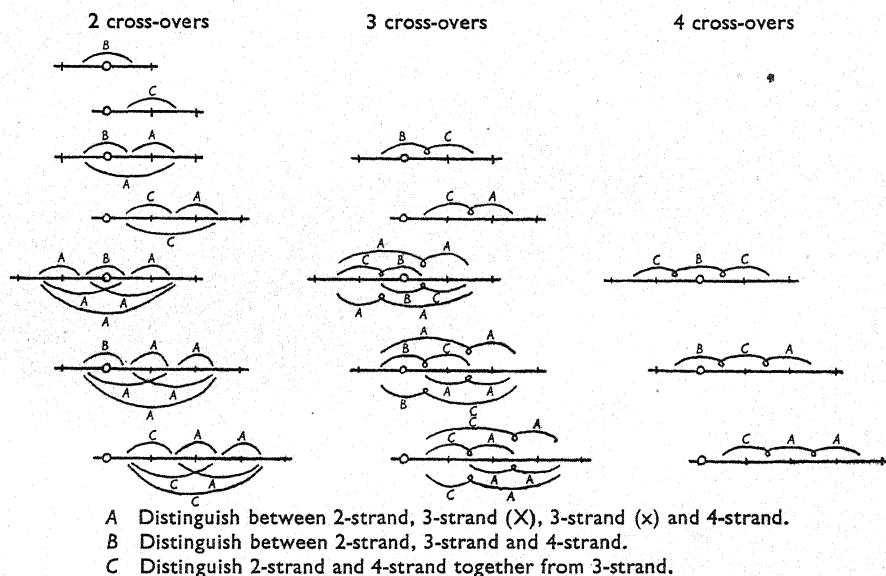


Fig. 4. Diagram to show the regions between which two-strand and four-strand double cross-overs give the same classes of spore arrangements, for various dispositions of two, three and four gene loci in relation to the centromere.

(ii) Between a region adjoining the centromere and another region in the same chromosome arm, all the genes being in the same arm, two-strand and four-strand double cross-overs cannot be distinguished, nor can the two kinds of three-strand (type C, Fig. 4).

(iii) Between a region adjoining the centromere and another region in the same arm, a third cross-over having occurred in the region adjoining the centromere in the other arm, then two-strand and four-strand double cross-overs are again indistinguishable, besides the two kinds of three-strand (type C).



(b) *Two cross-overs within one region*

Four-strand double cross-overs are detectable when the region is not bounded on one side by the centromere, but as the corresponding two-strand and three-strand double cross-overs cannot be detected, the four-strand doubles must not be counted when making comparisons.

In analysing the spore arrangements resulting from two cross-overs in different regions, the effect of a double cross-over within one of the regions is important, because three cross-overs, two in one of the regions and one in the other, can give the same spore arrangements as two cross-overs, one in each of the regions. When the double cross-over within one region is two-strand, whatever the cross-over

Table 5

+ — I — ○ — II — +

I and II	Class	3-st. in I or II
2-st.	5	7
3-st.	7	5 and 6
4-st.	6	7

○ — I — + — II — +

I and II	Class	3-st. in I or II
2-st. or 4-st.	4	7
3-st.	7	4

+ — I — ○ — II — + — III — +

II and III	Class	3-st. in II or III
2-st.	7	11 and 12
3-st. (X)	12	} 7 and 8
3-st. (x)	11	
4-st.	8	11 and 12

relationship between the two regions, the resulting spore arrangements are the same as for single cross-overs. When the double cross-over within one region is four-strand it is detectable, unless the region is bounded on one side by the centromere, in which case the resulting spore arrangements are again the same as for single cross-overs. When, however, the double cross-over within one region is three-strand, then two-strand and four-strand cross-over relationships between the two regions give the same spore arrangements as a simple three-strand double cross-over one in each region; whilst a three-strand cross-over relationship between the two regions gives the same spore arrangements as the simple two-strand and four-strand double cross-overs one in each region. Three examples of this are shown in Table 5.

This reversal of the spore arrangements resulting from two-strand and four-strand double cross-overs on the one hand and from three-strand on the other,

when a three-strand double cross-over occurs in one of the regions, has the following consequences:

(i) If the proportion of these double cross-overs which are really triple cross-overs with a three-strand double in one region, is small, then a difference from the expected 1 : 1 ratio of two-strand and four-strand to three-strand will merely be reduced in magnitude.

(ii) If a large proportion of the double cross-overs are really triples with a three-strand double in one region, then any difference from 1 : 1 in the proportion of two-strand and four-strand to three-strand may be quite obscured or even reversed.

(iii) Where two-strand and four-strand double cross-overs produce different spore arrangements (types A and B, Fig. 4), when these double cross-overs are really triples with a three-strand double in one region, the spore arrangements corresponding to two-strand and to four-strand will occur equally often. This is because the triple cross-over, three-strand in one region plus three-strand between

Table 6

I			II		III	
I	II	III	Class	3-st. in I	3-st. in II	3-st. in III
2-st.	2-st.		19	27 and 28	29	25
	3-st.		25	29 and 30	27 and 28	19
	4-st.		19	27 and 28	30	25
3-st.	2-st.		27 and 28	19 and 20	25 and 26	29 and 30
	3-st.		29 and 30	25 and 26	19 and 20	27 and 28
	4-st.		27 and 28	19 and 20	25 and 26	29 and 30
4-st.	2-st.		20	27 and 28	30	26
	3-st.		26	29 and 30	27 and 28	20
	4-st.		20	27 and 28	29	26

the two regions, three strands in all, gives the same spore arrangements as a two-strand double cross-over between the two regions; whilst three-strand plus three-strand, four strands in all, gives the same spore arrangements as a four-strand double cross-over between the two regions; and it is unlikely that three-strand plus three-strand, three in all, and three-strand plus three-strand, four in all, will not occur with equal frequencies. Hence, if a proportion of the double cross-overs are really triples with a three-strand double in one region, then any difference from 1 : 1 in the proportion of two-strand to four-strand will merely be reduced in magnitude, the ratio approaching equality the more closely as the proportion of triples is increased.

When there are three or four cross-overs in different regions, the effect of a double cross-over within one of the regions, is the same as its effect when there are only two cross-overs in different regions, i.e. two-strand and four-strand double cross-overs within a region do not matter, whilst three-strand cause a reversal of the spore arrangements resulting on the one hand from two-strand and four-strand and on the other hand from three-strand cross-over relationships between the region containing the three-strand double, and other regions. An example illustrating this is given in Table 6.

When there are two, three or four cross-overs in different regions and three-strand double cross-overs occur within two of the regions, the effect on the spore arrangements is more complex, but can be neglected unless the regions are very long.

#### CHIASMA INTERFERENCE

Chiasma interference means that the occurrence of one cross-over or chiasma reduces the chance of another in its neighbourhood. Considering two different regions of a chromosome, delimited by genes or by a gene and the centromere, the lengths of the regions in cross-over units, when divided by 50, give the frequency of crossing-over in the regions, since a map distance of 50 units is equivalent to an average frequency of one cross-over in the region per meiosis. Hence the product of the two distances, after dividing each by 50, gives the expected frequency of double cross-overs (one cross-over occurring in each region), assuming no interference. If the observed number of such double cross-overs is significantly less than the number calculated by this means, then there is positive chiasma interference. The observed frequency of double cross-overs must include 'doubles' within triples, quadruples, etc., if these involve cross-overs in the two appropriate regions, since cross-overs outside these two regions are not being considered. A distinction should be drawn between those cases in which the centromere lies between the two regions, and those in which both regions lie in the same arm, since there is evidence that the centromere acts as an interference-inhibitor. Between arms, therefore, one might expect no chiasma interference.

The product of three map lengths, after dividing each by 50, gives in the same way, the frequency of triple cross-overs, one cross-over in each of the regions, to be expected on the assumption of no interference; and similarly the frequencies of quadruple cross-overs can be calculated.

#### NUCLEAR PASSING

As mentioned above, the possibility that occasional nuclear passing takes place during the divisions in the ascus must not be neglected. Nuclear passing at the third division would frequently be detected if it occurred, as the spores would not be in identical pairs. Passing of the centre nuclei at anaphase of the second division, however, is less readily detected.

Consider the six spore arrangements that occur in asci showing segregation for a single gene pair such as + and -. The arrangements ++-- and --++ would be interchanged with +-+- and -+ -, if nuclear passing had occurred at the second division, whilst +-+- and -+ +- would be unaffected (see Fig. 1). Dividing the second division segregation asci (class 2, Table 1) into two subclasses, class 2A the alternate arrangements (+-+- and -+ +-) and class 2B the symmetrical arrangements (++-- and --++), then the effect of nuclear passing after the second division is to cause an interchange of asci between classes 1 and 2A, whilst class 2B is unaffected. If there is less than 66.7% second division segregation, class 2A will be smaller than class 1. Interchange will therefore increase 2A and decrease 1, the effect being greater the larger the disparity in size

of the two classes. Hence nuclear passing will cause genes with a small percentage of second division segregation to give more asci with the alternate arrangement (2A) than with the symmetrical arrangement (2B). However, unless a gene is available located very close to the centromere, a small percentage of nuclear passing is not easily detected by this means. For instance, if there is 5% nuclear passing, at least 300 asci showing segregation for a gene giving 10% second division segregation, will have to be analysed before the number in class 2A is likely to be significantly greater (5% probability) than the number in class 2B; and with a gene giving 20% second division segregation, at least 750 asci will have to be analysed. If there is 2% nuclear passing, the corresponding figure for a gene giving 5% second division segregation is 800 and for a gene giving 10% second division segregation, it is over 1500; if there is only 1% nuclear passing, the corresponding figure for a gene giving 5% second division segregation is over 2500. For a gene showing more than 66.7% second division segregation, class 2A will be larger than class 1 and interchange will render 2A smaller than 2B, but this will be even less readily detected.

In considering the effect of second division nuclear passing on the spore arrangements in asci showing segregation for two, three and four pairs of allelomorphs, it is convenient to divide up all the classes of spore arrangements in which at least one pair of allelomorphs segregated at the second division into two subclasses A and B, as was done in the case of asci showing segregation for a single gene pair. It is understood that when two or more pairs of allelomorphs have segregated at the second division, subclass A shows the alternate arrangement and B the symmetrical arrangement for the leftmost gene that shows second division segregation, that is, taken in the order *XY*, *XYZ* or *WXYZ* in conformity with

Tables 2-4. For instance,  $\frac{XxXx}{YyyY}$  belongs to class 7A (two gene loci), whilst

$\frac{XxxX}{YyYy}$  belongs to 7B.

Table 7 shows the effect of nuclear passing on the classes of spore arrangements in asci showing segregation for one, two, three or four pairs of allelomorphs. Most of the classes are grouped in threes as a concise form in which to express the interchanges of asci between the different classes. In each of these sets of three, asci belonging to subclass A of the class represented by the first figure will be interchanged with asci belonging to subclass A of the class represented by the second figure; likewise asci in B of the second class will be interchanged with B of the third class; and A of the third with B of the first. For example, with two gene loci, 3, 4, 7 means 3A interchanged with 4A, 4B with 7B and 7A with 3B.

The effects of occasional interchange of asci between these classes will be negligible unless the two classes concerned differ greatly in the number of asci which they contain. The size of the classes will depend on the map distances between the genes, so that every case must be considered individually. However, whether there are two, three or four linked genes, provided the distances between them are comparatively short, the classes of asci are likely to fall into three groups as regards size. The non-cross-over spore arrangements will be the largest class, then those due to single cross-overs will be moderately frequent and ascospore arrangements requiring two or more cross-overs will be relatively uncommon. It is the latter,



however, which give the data on chromatid interference, and it is clear that certain classes of arrangements amongst them may be increased in frequency by the interchange of asci with the non-cross-over class; and others may be increased, to a lesser extent, by interchange with the single cross-over classes of asci.

Table 7

No. of gene loci	Changes in classes of spore arrangements			
1	1 interchanged with 2 A		2 B unchanged	
2	1 interchanged with 5 A 2 interchanged with 6 A		5 B unchanged 6 B unchanged	
	3, 4, 7			
3	1 interchanged with 21 A 2 interchanged with 22 A 3 interchanged with 23 A 4 interchanged with 24 A		21 B unchanged 22 B unchanged 23 B unchanged 24 B unchanged	
	5, 19, 25 6, 20, 26	7, 15, 29 8, 16, 30	9, 13, 31 10, 14, 32	11, 17, 27 12, 18, 28
4	1 interchanged with 105 A 2 interchanged with 106 A 3 interchanged with 107 A 4 interchanged with 108 A 13 interchanged with 125 A 14 interchanged with 126 A 15 interchanged with 127 A 16 interchanged with 128 A		105 B unchanged 106 B unchanged 107 B unchanged 108 B unchanged 125 B unchanged 126 B unchanged 127 B unchanged 128 B unchanged	
	5, 103, 109 6, 104, 110 7, 99, 113 8, 100, 114 9, 97, 115 10, 98, 116 11, 101, 111 12, 102, 112 17, 123, 129 18, 124, 130 19, 119, 133 20, 120, 134 21, 117, 135	22, 118, 136 23, 121, 131 24, 122, 132 25, 77, 153 26, 78, 154 27, 79, 155 28, 80, 156 29, 73, 157 30, 74, 158 31, 75, 159 32, 76, 160 33, 81, 149 34, 82, 150	35, 83, 151 36, 84, 152 37, 65, 165 38, 66, 166 39, 67, 167 40, 68, 168 41, 61, 169 42, 62, 170 43, 63, 171 44, 64, 172 45, 69, 161 46, 70, 162 47, 71, 163	48, 72, 164 49, 89, 141 50, 90, 142 51, 91, 143 52, 92, 144 53, 85, 145 54, 86, 146 55, 87, 147 56, 88, 148 57, 93, 137 58, 94, 138 59, 95, 139 60, 96, 140

Considering first the interchange of asci with the non-cross-over class, whether there are two, three or four pairs of allelomorphs so long as the genes are dispersed on either side of the centromere, the effect is similar. From Table 7 the classes concerned are 5A (two loci), 21A (three loci) and 105A (four loci), the respective B subclasses being unaffected. Reference to Tables 2, 3, 4(b) and 4(c) shows that all these spore arrangements are due to two-strand double cross-overs across the centromere, the cross-overs occurring in the regions adjoining the centromere. Hence the major effect of second division nuclear passing will be an apparent increase in two-strand double cross-overs across the centromere. But this increase will occur only in the asci showing the alternate arrangement of the spores and not in those with the symmetrical arrangement. This gives the best test for second

division nuclear passing: a significant excess of class 5A over 5B with two genes one in each arm; of 21A over 21B with three genes, two in one arm and one in the

other; and, best test of all, an excess of 105A  $\begin{matrix} Ww & Ww \\ Xx & Xx \\ Yy & Yy \\ Zz & Zz \end{matrix}$  over 105B  $\begin{matrix} Ww & ww & W \\ Xx & xx & X \\ Yy & yy & Y' \\ Zz & zz & Z \end{matrix}$  with

four genes, two in each arm or three in one arm and one in the other. In the case of two, three or four genes all in one arm of the chromosome, classes 5 (two loci), 21 (three loci) and 105 (four loci) are produced by a single cross-over in the region adjoining the centromere. Hence interchange with the non-cross-over asci will not be detected so readily, the classes probably not differing very widely in magnitude.

Considering secondly the interchange of asci with the single cross-over classes, examination of Table 7 in conjunction with Tables 2-4 shows that the following classes will be affected: two genes, one in each arm: class 7; two genes in one arm: classes 4A and 7A; three genes, two in one arm and one in the other: classes 19A, 25A and 31; three genes in one arm: classes 13A, 19A, 25A and 31A; four genes, two in each arm: classes 41A, 103A, 109A, 115 and 169B; four genes, three in one arm and one in the other: classes 97A, 103A, 109A, 115A and 169; four genes in one arm: classes 61A, 97A, 103A, 109A, 115A and 169A. Expressing these classes in terms of the cross-overs that normally produce them, it is possible to group them together, irrespective of the number of pairs of allelomorphs. The following cross-overs will appear to be increased in frequency:

- (i) Three-strand double cross-overs across the centromere, the cross-overs occurring in regions adjoining the centromere on either side.
- (ii) All double cross-overs between a region adjoining the centromere and any other region in the same chromosome arm, all the genes being in the same arm.
- (iii) Certain triple cross-overs consisting of a two-strand double cross-over across the centromere, the cross-overs occurring in regions adjoining it on either side, and a third cross-over taking place in any other region.

Thus the effect of second division nuclear passing on chromatid interference data will be to add to two-strand double cross-overs immediately across the centromere, and to a lesser extent to add to the corresponding three-strand double cross-overs; four-strand double cross-overs across the centromere and all cross-overs between other regions will be unaffected.

#### PLANNING AN EXPERIMENT TO TEST FOR CHROMATID INTERFERENCE

Three or four genes are the best numbers to use, as with only two genes very large numbers of asci would need to be analysed to give significant results, whilst to use more than four genes is impracticable since many of the gene combinations would almost certainly be indistinguishable. Using three or four genes all in one arm of the chromosome is to be avoided if possible, since then two-strand and four-strand double cross-overs are so often indistinguishable and second division nuclear passing less easily detected. In the sex chromosome, where use can be made of the

sex gene, the ideal arrangement for studying cross-overs across the centromere is four genes, two in each arm, and for cross-overs in one arm, four genes, three in one arm and one in the other. In the autosomes, however, with as many as four genes all the gene combinations may not be distinguishable, and then three genes, two in one arm and one in the other, is the best arrangement.

The optimum distance between loci is probably about 15 units and a region should never exceed 30 units. If the regions are too short, a very large number of asci will have to be analysed before significant results are obtained. If the regions are too long a proportion of the double cross-overs will really be triple cross-overs with a three-strand double within one region, and in consequence any chromatid interference will be less easily detected, and in the case of the proportion of two-strand and four-strand to three-strand, the result may be reversed. The frequency of four-strand double cross-overs within regions is a guide to the frequency of the corresponding three-strand double cross-overs, and hence to the validity of data on chromatid interference.

#### SUMMARY

*Neurospora sitophila* and *N. crassa* are heterothallic fungi in which a single diploid nucleus in the young ascus divides three times in succession, the first two divisions constituting meiosis and the third a mitosis. Eight spores are formed round the eight nuclei, and by removing the spores in order from individual asci, not only can all four products of a single meiosis be isolated and grown to maturity, but first and second division segregation can be distinguished. Hence, genes can be mapped in relation to the centromere as well as in relation to one another, and from analysis of the character and order of the spores in asci showing segregation for a number of linked genes, the position of the cross-overs that occurred at meiosis can be determined. Moreover, this gives a genetic test for chromatid interference, for two-strand, three-strand and four-strand relationships between cross-overs are frequently distinguishable by the spore arrangements they produce. Tables are given showing the cross-overs associated with all the possible arrangements of the spores in asci showing segregation for two, three or four linked genes, with various dispositions of the genes in relation to the centromere. The effect of possible nuclear passing after the second division is considered, and the best positions for the genes in testing for chromatid interference are described.

## II. LINDEGREN'S RESULTS WITH *NEUROSPORA CRASSA*

### INTRODUCTION

Lindegren has mapped five genes on the sex chromosome of *Neurospora crassa* and three on the second chromosome. Those on the sex chromosome, besides the sex gene, are the genes *gap*, *crisp*, *pale* and *dirty*, and on the second chromosome the genes *peach*, *tuft* and *fluffy*; these represent mutant forms of the fungus, differing from the wild type chiefly in the distribution, abundance and colour of the conidia. The wild type or *normal N. crassa* produces abundant orange conidia in a rather

dense mass. The chief characters by which these seven mutants are distinguished are the following:

*Gap, G.* Conidia in a few scattered clusters on long non-conidial hyphae.

*Crisp, C.* Conidia very densely massed and much more brilliant in colour than *normal*.

*Pale, P.* Conidia about half as abundant as *normal*, lighter coloured and somewhat clumped.

*Dirty, D.* Conidia replaced by a yellowish exudate from the abundant aerial mycelium.

*Peach, Pe.* Conidia of a delicate peach colour, slightly fewer and less dense than *normal*.

*Tuft, Tu.* Conidia in large bunches at the top of the white aerial mycelium.

*Fluffy, F.* Conidia absent, but an abundance of white aerial mycelium.

Amongst the many possible double mutants, *crisp-pale* differs from *crisp* in that the conidia are slightly less abundant and lighter in colour and *gap-dirty* has the conidia replaced by a rusty brown exudate and the growth is much poorer than in either *gap* or *dirty*.

Lindegren has analysed a large number of asci showing segregation for various combinations of these genes. Altogether five papers have been published giving the results of these analyses. The most important of these papers are the fourth (1937) in which details of 419 asci showing segregation for sex, *gap*, *crisp* and *pale* are given, and the fifth (1939) containing an analysis of 278 asci showing segregation for *peach*, *tuft* and *fluffy*. These analyses provide data on chromatid and chiasma interference in crossing-over. A study of Lindegren's papers has shown that the following errors have been made in the inferences drawn from the data:

(1) In comparing the frequencies of two-strand, three-strand and four-strand relationships between cross-overs, four-strand double cross-overs, both in one interval, have been wrongly included, for the corresponding two-strand and three-strand double cross-overs cannot be detected.

(2) Many spore arrangements due equally likely to two-strand or to four-strand double cross-overs have been recorded as due to two-strand doubles.

(3) In calculating the expected frequency of double cross-overs, assuming no chiasma interference, the two appropriate map distances have to be multiplied (after dividing each by 50). The observed frequency of double cross-overs must include 'doubles' within triples and quadruples, since cross-overs outside the two regions under consideration are being neglected. These 'doubles' within triples and quadruples have been omitted, leading to the erroneous conclusion that double cross-overs symmetrically placed about the centromere are abnormally frequent.

(4) Incompletely analysed asci have been omitted without allowing for the fact that they were probably not a random sample. Asci showing second division segregation for a number of genes are much more likely to be incompletely scored than those in which segregation is predominantly at the first division. If, for instance, the spores in one half of an ascus have failed to germinate, if not more than one pair of allelomorphs has segregated at the second division, the ascus can be put into its correct class as regards spore arrangement. If, however, two or more pairs of allelomorphs have segregated at the second division, it is impossible to



determine whether these genes were in their original combination or whether they were recombined in the missing half of the ascus, and so the class to which the ascus belongs is uncertain. If such asci are discarded, the results will become biased. If at all frequent such incomplete asci must be recorded, together with the possible classes to which they might belong.

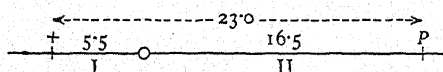
(5) Attempts have been made to correct map distances within one arm of a chromosome by means of the cross-overs deduced from the arrangements of the ascospores. Such correction is only possible between adjacent genes on either side of the centromere.

These errors have necessitated a recalculation of the results to be deduced from Lindgren's analyses of asci of *N. crassa*. Each of his sets of data on the frequencies of different ascospore arrangements will be considered in turn.

### RESULTS

In the first paper (1933, p. 149), an analysis is given of 109 asci showing segregation for the two genes, *sex* and *pale*, in opposite arms of the chromosome. The parental cross was  $+P \times -p$  ( $P = \text{pale}$ ,  $p = \text{normal}$ ), so that to put the spore arrangements into the seven classes possible with two genes, as shown in Table 2,  $+$  is replaced by  $X$ ,  $-$  by  $x$ ,  $P$  by  $Y$  and  $p$  by  $y$ . The 109 asci are then found to be distributed amongst the classes with the frequencies shown in the second column of Table 8, class 5 not being represented. All the asci within each class must have

Table 8



Class (2 genes)	Asci	2nd div. seg.		Recomb. ( $\times 2$ ) + and P	Simplest cross-overs	Cross- overs + to P
		+	P			
1	62	0	0	0	Non-cross-over	0
4	10	10	0	10	1 in I	10
3	34	0	34	34	1 in II	34
7	1	1	1	1	3-st. } I and II	2
6	1	1	1	2	4-st. }	2
2	1	0	0	2	4-st. in I or II	2
Total	109	12	36	49		50
Percentage		11.0	33.0	45.0		45.9
Map distance		5.5	16.5	22.5		23.0

shown segregation at the same division for each pair of allélomorphs and have a constant percentage of recombination; for instance, asci in class 4 showed segregation at the second division for  $+$ , at the first division for  $P$  and gave 50% recombination. The third and fourth columns in Table 8 give the number of asci showing second division segregation for  $+$  and for  $P$  respectively. The totals are given at the bottom and the percentages of second division segregation halved to give the map distances of the genes from the centromere. The fifth column in

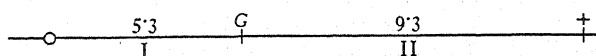
Table 8 indicates the recombination that has occurred between  $+$  and  $P$ . In any individual ascus there are three possibilities: all four products of meiosis, i.e. pairs of spores, may show the original combination of the genes; or two may be original combination types and two recombination types; or all four may be recombined. This recombination might be described in terms of the asci as a whole, when the three possibilities would be denoted by 0,  $\frac{1}{2}$  and 1, respectively; but it is more convenient to record the recombination in terms of half-asci and denote the alternatives by 0, 1 and 2, respectively, and this is the method adopted throughout this paper. Hence, the sum of the figures in the fifth column of Table 8, after expression as a percentage, must be halved to give the percentage of recombination and the map distance. The simplest cross-overs to give each class of spore arrangement are entered from Table 2 in the sixth column of Table 8. In the final column, these cross-overs are used to correct the map distance from  $+$  to  $P$  by recording all the cross-overs known to have occurred in the interval. The analysis gives very few data on interference. Double cross-overs, one in each region, show 0 two-strand, 1 three-strand, 1 four-strand. The expected frequency of all such double cross-overs, if there is no chiasma interference, is  $0.33 \times 0.11 \times 109 = 4.0$ . The observed frequency is 2. As regards passing of the two centre nuclei after the second division in the ascus, there are 62 asci in class 1, which would have given class 5 asci had the nuclei passed. The absence of any asci in class 5 shows that nuclear passing is rare if it occurs at all.

In the second paper (1936*a*, p. 250), an analysis of 178 asci showing segregation for *gap* and sex, both in the same arm, is given.  $+G$  was crossed with  $-g$ , and so putting  $G \equiv X$ ,  $g \equiv x$ ,  $+$   $\equiv Y$  and  $- \equiv y$ , the six rows of asci in the table can be converted to the classes given in Table 2. Class 6 is not represented. The results are shown in Table 9, the figures in the third, fourth and fifth columns giving the numbers of asci showing second division segregation and of half-asci showing recombination, from which the map distances are calculated below. The simplest cross-overs to give each class of spore arrangement are shown in the last column, taken from Table 2. It is impossible to correct the map distance from the centromere to  $G$  or from  $G$  to  $+$  by means of these cross-overs, since both genes are in the same arm. Neglecting the four asci due to four-strand double cross-overs in the interval between  $G$  and  $+$ , since the corresponding two-strand and three-strand doubles cannot be detected, the asci give 3 two-strand or four-strand and 2 three-strand double cross-overs. The expected total number of such doubles is  $0.107 \times 0.185 \times 178 = 3.5$ , assuming no chiasma interference; the observed number is 5. These data on interference are again not large enough for any conclusions to be drawn. On page 252 of the same paper, 115 of these 178 asci are analysed for the gene *dirty*,  $D$ . This gene, however, is located more than 50 units from the centromere in the opposite arm to  $G$  and  $+$ , so that the interval is much too long for the cross-overs to be analysed at all accurately.

The third of Lindegren's papers (1936*b*, p. 255) gives the results obtained from seventy-eight asci showing segregation for sex, *crisp* and *pale*, the sex gene being in one arm and *crisp* and *pale* in the other. It is important to note that the parental cross was  $+crisp \times -pale$ , so use can be made of Table 3 by putting  $+$   $\equiv X$ ,  $- \equiv x$ ,  $C \equiv Y$  and  $c \equiv y$ , but  $P \equiv z$  and  $p \equiv Z$ . Fourteen of the thirty-two

possible classes are represented, and they are shown in Table 10, together with the numbers of asci showing second division segregation for each of the three genes and the numbers of half-asci showing recombination, from which all the map distances are calculated at the bottom of the table. The simplest cross-overs are shown, taken from Table 3, and in the final column the cross-overs known to have occurred between + and *C* are listed, giving a better estimate of the true map distance between these two genes. In comparing the frequencies of two-strand, three-strand and four-strand relationships, the four asci one in each of classes 2, 4, 6 and 14, must be omitted since the four-strand double cross-overs associated with them occurred within regions. Counts of the remainder give the following results: Across the centromere there were 4 two-strand, 1 three-strand and 0 four-strand; within the *pale* arm there was 1 two-strand, 0 three-strand, 1 four-strand and 1

Table 9

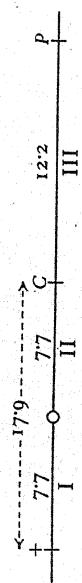


Class (2 genes)	Asci	2nd div. seg.		Recomb. ( $\times 2$ ) <i>G</i> and +	Simplest cross-overs
		<i>G</i>	+		
1	135	0	0	0	Non-cross-over 1 in I 1 in II 2-st. or 4-st. } I and II 3-st. 4-st. in II
5	14	14	14	0	
3	20	0	20	20	
4	3	3	0	3	
7	2	2	2	2	
2	4	0	0	8	
Total	178	19	36	33	
Percentage		10.7	20.2	18.5	
Map distance		5.3	10.1	9.3	

either two-strand or four-strand. The excess of two-strand relationships across the centromere at once suggests nuclear passing, but class 21, the class to which non-cross-over asci would be transferred by passing of the centre nuclei after the second division, is represented by only a single ascus. The other three two-strand cross-over relationships between arms, due to asci in classes 15 and 19, are most unlikely to be due to nuclear passing. The data on chiasma interference derived from this analysis of 78 asci are shown in Table 14*a* and *b*, the observed number of double cross-overs including 'doubles' within the triple cross-over, but again the figures are too small to allow conclusions to be drawn from them.

In Lindegren's fourth paper (1937, p. 106) an analysis of 419 asci showing segregation for four genes is given, *sex* and *gap* in one arm, *crisp* and *pale* in the other. In this experiment +*gap pale* was crossed with -*crisp*, so use can be made of Table 4 by putting +  $\equiv W$ , -  $\equiv w$ , *G*  $\equiv X$ , *g*  $\equiv x$ , *C*  $\equiv y$ , *c*  $\equiv Y$ , *P*  $\equiv Z$  and *p*  $\equiv z$ . Twenty-one of the 172 possible classes are represented and their frequencies are given in Table 11. Three asci in Lindegren's table had to be omitted as their class was doubtful. The omission of such incomplete asci is regrettable since the results are likely to be affected thereby, but not knowing in what respects they were incom-

Table 10



Class (Lindegren)	Class (3 genes)	Asci	2nd div. seg.			Recomb. ( $\times 2$ )			Simplest cross-overs	Cross-overs + to C
			+	C	P	+ and C	+ and P	C and P		
a d c b	1	45	0	0	0	0	0	0	Non-cross-over	0
	13	6	6	0	0	6	6	0	1 in I	6
	9	7	0	7	7	7	7	0	1 in II	7
	5	9	0	0	9	0	9	9	1 in III	0
f i e g n h k	21	1	1	1	1	0	0	0	2-st. I and II	2
	31	1	1	1	1	1	1	0	3-st. I and III	2
	15	2	2	0	2	2	0	2	2-st. I and III	2
	7	1	0	1	0	1	0	1	2-st. II and III	1
	8	1	0	1	0	1	2	1	4-st. I and III	1
	4	1	0	0	0	2	2	0	4-st. in I or II	2
	4	1	0	0	0	0	2	2	4-st. in III	0
	2	1	0	0	0	0	2	2	2-st. + 2-st. or 4-st., I, II and III	2
l m j	10	1	1	1	0	0	1	1	4-st. in I or II, 1 in III	2
	6	1	0	0	1	2	1	1	1 in I, 4-st. in III	2
	14	1	1	0	0	1	1	2		1
Total		78	12	12	21	23	32	19		28
Percentage			15.4	15.4	26.9	29.5	41.0	24.4		35.9
Map distance			7.7	7.7	13.5	14.7	20.5	12.2		17.9



plete it is impossible to allow for them. In columns 3-12 of Table 11 are given the numbers of asci showing second division segregation for each of the genes, and the numbers of half-asci showing recombination, the appropriate map distances being deduced at the bottom by halving the totals expressed as percentages. There was more recombination between *G* and *P* than between + and *P* largely owing to the excess of asci in class 65. In the final column the simplest cross-overs are given, taken from Table 4*b*. '3-st. (+)' means the strand involved at the second cross-over but not at the first was derived from the + parent, '3-st. (-)' from the - parent. As there were no four-strand double cross-overs within regions, it is impossible to correct the distance from *G* to *C* by means of the cross-overs deduced from the spore arrangements.

Counting the frequencies of two-strand, three-strand and four-strand cross-over relationships gives the following results: Across the centromere the double cross-over asci include 12 two-strand, 8 three-strand, 4 four-strand, the triples provide 5 two-strand, 2 three-strand, 1 four-strand and the quadruples 3 two-strand and no three-strand or four-strand; hence the total is 20 *two-strand*, 10 *three-strand*, 5 *four-strand*. Comparison with the expected proportion of 1 : 2 : 1 indicates  $\chi^2 = 19.3$ , the probability for which, for 2 degrees of freedom, is  $\ll 0.01$ , so that the results are significantly different from expectation assuming no chromatid interference. Within arms, where two-strand and four-strand can be distinguished, there are no two-strand or three-strand but 3 four-strand (from classes 32 and 86); where two-strand and four-strand are indistinguishable, there are 7 two-strand or four-strand, 4 three-strand. Hence the total within arms is 10 *two-strand or four-strand*, 4 *three-strand*, which is not significantly different from 1 : 1. The grand total for all regions is 35 *two-strand or four-strand*, 14 *three-strand*, which is significantly different from 1 : 1. The chiasma interference data are shown in Table 14*a* and *b*, the observed numbers of double cross-overs including 'doubles' within the triples and quadruples, and the observed numbers of triples including 'triples' within the quadruples. There is a marked excess of double cross-overs between arms and the excess is proportionately even greater with the triples and quadruples. Within arms, the observed numbers are only slightly in excess of expectation and are probably not inconsistent with the existence of positive chiasma interference. As regards nuclear passing, there are 290 asci in class 1 which would have been converted instead into class 105A if the centre nuclei had passed after the second division. There are only two asci in class 105; it is impossible to tell from Lindegren's published data whether these showed the alternate arrangement of the pairs of spores (class 105A) or the symmetrical arrangement (105B), but obviously the number of asci scored as class 105 but really belonging to class 1 cannot exceed 2; it is clear that if nuclear passing occurs at all it probably occurs in less than 1% of asci. The only other large class is 5 with 52 asci; passing here would have given classes 103 and 109, in each of which there is only a single ascus. 16 of the 20 two-strand cross-over relationships across the centromere are due to the asci in classes 65, 41, 169, 37 and 45. All of these are most unlikely to have resulted from nuclear passing, which could not possibly explain an excess in these classes. Classes 105, 103 and 109 are the only classes in which an excess might be due to nuclear passing.

Table II

+ ————— +											
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The fifth paper (1939) contains an analysis of 278 asci showing segregation for *peach*, *tuft* and *fluffy*, all in the same arm of the second chromosome. In the table on p. 3 of Lindegren's paper, the various spore arrangements are unfortunately only given in terms of the cross-overs that produced them, so that it is impossible to check that the cross-overs have been deduced correctly. Taking *Pe* as *X*, *Tu* as *Y*, *F* as *Z*, the classes of spore arrangements given in the first column of Table 12 have therefore had to be determined from the cross-overs in the last column, by using Table 3 in reverse manner from normal. The numbers of asci showing second division segregation and of half-asci showing recombination have then been deduced from the assumed spore arrangements. Omitting the two four-strand double cross-overs within a region, the frequencies of two-strand, three-strand and four-strand cross-over relationships are 5 *two-strand*, 3 *three-strand*, 2 *four-strand* and, where two-strand and four-strand cannot be distinguished, 15 *two-strand or four-strand*, 14 *three-strand*. The grand total is therefore 22 *two-strand or four-strand*, 17 *three-strand*. None of these results is significantly different from expectation assuming no chromatid interference, but the deviation from expectation is in the same direction as in the sex chromosome. As regards chiasma interference data, Lindegren's figures are correct and are included in Table 14*b*; they indicate positive chiasma interference.

#### DISCUSSION

The chromatid interference data deduced from Lindegren's analyses of asci are summarized in Table 13*a* and *b*. The total for cross-over relationships between arms is 24 *two-strand*, 12 *three-strand*, 6 *four-strand*. Comparison with the expected proportion of 1:2:1, assuming no chromatid interference gives  $\chi^2 = 23.1$ , the probability for which, for 2 degrees of freedom, is  $\ll 0.01$ . Hence the results are significantly different from expectation. As already explained only five of the two-strand cross-over relationships belong to classes in which an excess could be the result of passing of the centre nuclei after the second division, and there is no evidence at all that such nuclear passing ever occurs. Hence the excess of two-strand relationships and/or shortage of three-strand and four-strand between arms would seem to be explicable only in terms of chromatid interference. Within arms, where two-strand and four-strand can be distinguished the total is 6 *two-strand*, 3 *three-strand*, 6 *four-strand*, and where two-strand and four-strand are indistinguishable, the total is 26 *two-strand or four-strand*, 20 *three-strand*. Neither of these results is significantly different from expectation, but grouping them together the total for within arms is 38 *two-strand or four-strand*, 23 *three-strand*, which is almost significantly different from 1:1, for the probability is 0.055. As the deviation is in the same direction as between arms, this result is very suggestive that within arms there is also a shortage of three-strand compared with two-strand plus four-strand. The grand total for all regions is 68 *two-strand or four-strand*, 35 *three-strand*, which is markedly significantly different from the proportion of 1:1 expected on the assumption of no chromatid interference.

The chiasma interference data are summarized in Table 14*a* and *b*. Taken as a whole there is a marked excess of multiple cross-overs between arms, and especially of triples and quadruples. Passing of the centre nuclei after the second

Table 12

—○—	11.3	Pe	8.5	Tu	18.9	F
	I		II		III	

Class (3 genes)	Asci	2nd div. seg.			Recomb. ( $\times 2$ )			Simplest cross-overs
		Pe	Tu	F	Pe and Tu	Pe and F	Tu and F	
I	104	0	0	0	0	0	0	Non-cross-over 1 in I 1 in II 1 in III
21	34	34	34	34	0	0	0	
9	26	0	26	26	26	26	0	
5	76	0	0	76	0	76	76	
13	5	5	0	0	5	5	0	2-st. or 4-st. } I and II 3-st. } 2-st. or 4-st. } I and III 3-st. } 2-st. } II and III 3-st. } 4-st. } 4-st. in III
31	6	6	6	6	6	6	0	
19	9	9	9	0	0	9	9	
25	6	6	6	6	0	6	6	
7	2	0	2	0	2	0	2	
11 and 12	3	0	3	3	3	3	3	
8	2	0	2	0	2	4	2	
2	2	0	0	0	0	4	4	
15	1	1	0	1	1	0	1	
29	2	2	2	2	2	0	2	
Total	278	63	90	154	47	139	105	
Percentage		22.6	32.4	55.4	16.9	50.0	37.9	
Map distance		11.3	16.2	27.7	8.5	25.0	18.9	



Table 13a. *Between arms*

Source	Genes	2-st.	3-st.	4-st.
1933	$+ .P$	0	1	1
1936b	$+ .C P$	4	1	0
1937	$+ G . C P$	20	10	5
Total		24	12	6

Table 13b. *Within arms*

Source	Genes	2-st.	3-st.	4-st.	2-st. or 4-st.	3-st.
1936a	$.G +$	—	—	—	3	2
1936b	$+ .C P$	1	0	1	1	0
1937	$+ G . C P$	0	0	3	7	4
1939	$.Pe Tu F$	5	3	2	15	14
Total		6	3	6	26	20

Table 14a. *Between arms*

No. of cross-overs	Source	Regions	Calc.	Obs.	Obs./Calc.
2	1933	$+ .$ and $. P$	4.0	2	0.5
	1936b	$+ .$ and $. C$	1.9	3	1.6
		$+ .$ and $C P$	2.9	3	1.0
		$+ G$ and $. C$	2.7	10	3.7
	1937	$+ G$ and $C P$	7.0	25	3.6
		$G .$ and $. C$	2.3	11	4.8
3	1936b	$+ ., . C$ and $C P$	0.5	1	2.2
		$+ G, G .$ and $. C$	0.2	6	29.7
		$+ G, G .$ and $C P$	0.5	4	7.8
	1937	$+ G, . C$ and $C P$	0.5	5	9.7
		$G ., . C$ and $C P$	0.4	5	11.5
4	1937	$+ G, G ., . C$ and $C P$	0.04	3	78.6

Table 14b. *Within arms*

No. of cross-overs	Source	Regions	Calc.	Obs.	Obs./Calc.
2	1936a	$. G$ and $G +$	3.5	5	1.4
	1936b	$. C$ and $C P$	2.9	3	1.0
	1937	$. G$ and $G +$	2.7	7	2.6
		$. C$ and $C P$	5.9	7	1.2
		$. Pe$ and $Pe Tu$	10.7	14	1.3
	1939	$. Pe$ and $Tu F$	23.8	18	0.8
		$Pe Tu$ and $Tu F$	17.9	10	0.6
3	1939	$. Pe, Pe Tu$ and $Tu F$	4.0	3	0.7

division cannot explain this result, for, as shown above, there is not an excess of asci in the few classes in which an excess could be due to nuclear passing. It would seem that the negative chiasma interference across the centromere is associated with the positive chromatid interference, the excess of double cross-overs between arms in fact being accounted for by the excess of two-strand cross-over relationships. Within arms, observed and calculated double and triple cross-overs are approximately equal, but the data are insufficient and the results are probably not inconsistent with positive chiasma interference.

The existence of negative chiasma interference (i.e. coincidence greater than unity) across the centromere agrees with the cytological work of Newcombe (1941) on *Trillium erectum* and certain data on the third chromosome of *Drosophila* (see Newcombe). That one cross-over should actually favour the formation of a second implies some modification of Darlington's theory of crossing-over (Darlington, 1935), which relates chiasma formation to a torsion in the paired threads.

Genetic evidence relating to chromatid interference in organisms other than *Neurospora* is very slight. In the sex chromosomes of male *Drosophila* a two-strand double cross-over occurs regularly (Darlington, 1934). The evidence from 'attached X's' and 'ring X' in *Drosophila* is somewhat conflicting (Mather, 1933; Bonnier & Nordenskiöld, 1937; Morgan, 1933). In female *Bombyx* there is an indication of an excess of two-strand double cross-overs, from the correlation in the percentages of second division segregation of different parts of the sex chromosome, which themselves show no linkage (Mather, 1935). In *Viola* (Clausen, 1926), *Pisum* (Emerson & Rhoades, 1933) and certain mice (Fisher & Mather, 1936), significantly more than 50% recombination has been recorded, implying an excess of four-strand double cross-overs.

Cytological evidence concerning chromatid interference can be obtained either by direct observation in favourable material at diplotene, metaphase I or anaphase I of meiosis, or from the numbers of first and second division bridges and fragments in inversion hybrids. Direct observation has indicated an excess of two-strand and four-strand relationships, grouped together (compensating), over three-strand (non-compensating) in *Stenobothrus* (Darlington & Dark, 1932), *Melanoplus* (Hearne & Huskins, 1935) and *Trillium* (Huskins & Newcombe, 1941). The evidence from inversion hybrids is more uncertain, but there are indications that two-strand and four-strand relationships (grouped together) are more frequent than three-strand in *Chorthippus* (Darlington, 1936) and *Fritillaria* (Frankel, 1937); equally frequent in *Paeonia* (Dark, 1936); and less frequent than three-strand in *Tulipa* (Upcott, 1937).

Considering the genetical and the cytological data relating to chromatid interference as a whole, there is a suggestion that an excess of two-strand cross-over relationships, such as appears to exist in *Neurospora*, may be fairly widespread. The simplest explanation, assuming Darlington's theory of the mechanism of crossing-over to be in the main correct, is that one chromatid, by its method of formation, is thinner and more fragile than the other and so tends to break more often (Mather, 1938).

All Lindegren's published data on the percentages of second division segregation are given in Table 15a for the genes of *N. crassa* that have been mapped,

and the percentages of recombination between them are given in Table 15*b*. Maps of the sex chromosome and of the second chromosome, based on the mean values from these data, are shown in Fig. 5. All map distances derived from the 178 asci analysed in the second paper (1936*a*) are consistently higher than the average, and

Table 15*a*. Distances from centromere

Gene	Source	Asci	2nd div. asci	% 2nd	Dist.
+	1933	449	58	12.9	6.5
	1936 <i>a</i>	178	36	20.2	10.1
	1936 <i>b</i>	80	13	16.3	8.1
	1937	419	55	13.1	6.6
	Total	1126	162	14.4	7.2
G	1936 <i>a</i>	188	23	12.2	6.1
	1937	419	31	7.4	3.7
	Total	607	54	8.9	4.4
C	1936 <i>b</i>	88	14	15.9	8.0
	1937	419	31	7.4	3.7
	Total	507	45	8.9	4.4
P	1933	109	36	33.0	16.5
	1936 <i>b</i>	88	23	26.1	13.0
	1937	419	99	23.6	11.8
	Total	616	158	25.6	12.8
D	1936 <i>a</i>	115	72	62.6	31.3
	1936 <i>b</i>	88	56	63.6	31.8
	Total	203	128	63.0	31.5
Pe	1939	278	63	22.6	11.3
Tu	1939	278	90	32.4	16.2
F	1933	108	68	61.8	30.9
	1939	278	154	55.4	27.7
	Total	386	222	57.5	28.8

all those derived from the 419 asci in the fourth paper (1937) consistently below the average. It would be interesting to know if meiosis took place at the same temperature in both cases. 1126 asci have been analysed for sex and in 162 of them the

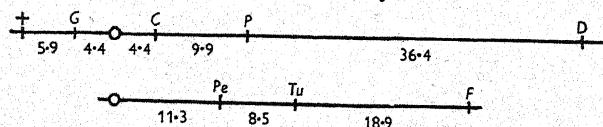


Fig. 5. Maps of the sex chromosome and of the second chromosome of *Neurospora crassa*, based on the whole of Lindegren's published data.

sex gene showed segregation at the second division; this gives 14.4% second division segregation and a map distance of 7.2 between the sex gene and the centromere. This distance however can be corrected through the use of the intermediate gene *gap*. 597 asci have given 5.9% recombination between sex and *gap*,

Table 15b. Distances between genes

Interval	Source	Asci	Recomb. ( $\times 2$ )	% Recomb. ( $\times 2$ )	Dist.
+ to <i>G</i>	1936a	178	33	18.5	9.3
	1937	419	37	8.8	4.4
	Total	597	70	11.7	5.9
+ to <i>C</i>	1936b	80	23	28.8	14.4
	1937	419	75	17.9	9.0
	Total	499	98	20.0	10.0
+ to <i>P</i>	1933	109	49	45.0	22.5
	1936b	78	32	41.0	20.5
	1937	419	120	28.6	14.3
	Total	606	201	33.2	16.6
+ to <i>D</i>	1936a	105	85	81.0	40.5
	1936b	80	62	77.5	38.8
	Total	185	147	79.4	39.7
<i>G</i> to <i>C</i>	1937	419	42	10.0	5.0
<i>G</i> to <i>P</i>	1937	419	121	28.9	14.4
<i>G</i> to <i>D</i>	1936a	115	91	79.1	39.6
<i>C</i> to <i>P</i>	1936b	78	19	24.4	12.2
	1937	419	79	18.9	9.4
	Total	497	98	19.7	9.9
<i>C</i> to <i>D</i>	1936b	88	65	73.9	36.9
<i>P</i> to <i>D</i>	1936b	88	64	72.7	36.4
<i>Pe</i> to <i>Tu</i>	1939	278	47	16.9	8.5
<i>Pe</i> to <i>F</i>	1939	278	139	50.0	25.0
<i>Tu</i> to <i>F</i>	1939	278	105	37.9	18.9

and 607 asci have given 8.9% second division segregation, equivalent to a map distance of 4.4 between *G* and the centromere. Hence the best estimate of the distance of the sex gene from the centromere is  $4.4 + 5.9 = 10.3$ .

## SUMMARY

A recalculation of the results to be derived from Lindegren's analyses of asci of *Neurospora crassa* showing segregation for a number of linked genes, leads to the following conclusions:

1. Between the two arms of the sex chromosome there is a marked excess of two-strand cross-over relationships compared with three-strand and four-strand, i.e. positive chromatid interference.
2. In the same region there is also a marked excess of double, triple and quadruple cross-overs, i.e. negative chiasma interference.



3. Within chromosome arms, insufficient data have been collected to give significant results, but there is a suggestion of an excess of two-strand and four-strand cross-over relationships (grouped together) compared with three-strand.

4. In the same regions, the data are probably consistent with positive chiasma interference.

5. There is no evidence to suggest that passing of the centre nuclei after the second division in the ascus ever occurs; there is good evidence that such passing must at least be rare and take place in less than 1% of asci. In any case, however frequently such passing might have occurred, it could not explain the positive chromatid interference and the negative chiasma interference across the centromere.

### III. THE SEX CHROMOSOME OF *NEUROSPORA SITOPHILA*

#### INTRODUCTION

The genetic study of *Neurospora sitophila* was begun by Wilcox (1928) who dissected six asci by hand. In one of these insufficient spores germinated to determine the division at which segregation for sex occurred, but in the remaining five segregation was at the second division. Dodge (1929) reported a mutant *albinistic* which produced very pale fluffy aerial mycelium and no conidia, instead of the masses of orange conidia of the *normal* or wild type. The dissection of fourteen asci by Dodge (1930), of fifteen by Lindegren (1932) and of twenty-five by Aronescu (1933) are the only further analyses that have been made. They gave the results shown in Table 16, the figures in the columns headed '2nd' giving the numbers of asci showing second division segregation. Altogether fifty-nine asci have been analysed for sex, of which thirty-two had segregated at the second division, or 54.2%, giving an uncorrected map distance of 27.1 between the sex gene and the

Table 16

Source	Sex			Albinistic			Half-asci	
	Asci	2nd	% 2nd	Asci	2nd	% 2nd	Orig.	Recomb.
Wilcox (1928)	5	5	100	—	—	—	—	—
Dodge (1930)	14	8	57.1	7	4	57.1	7	7
Lindegren (1932)	15	7	46.7	—	—	—	—	—
Aronescu (1933)	25	12	48.0	25	4	16.0	—	—
Total	59	32	54.2	32	8	25.0	7	7

centromere. Thirty-two asci have been analysed for *albinistic*, of which eight had segregated at the second division, giving 25% second division segregation and indicating that the gene for *albinistic* is 12.5 units from the centromere of its chromosome. There is no evidence of linkage between sex and *albinistic*. The asci analysed by Dodge gave seven original combinations and seven recombinations (counting a pair of + *albinistic* cultures and their complement, a pair of — *normal* cultures, as one, i.e. counting half-asci). Aronescu, unfortunately, does not record data on recombination. Hence, although it is almost certain that the gene for *albinistic* is

not in the same chromosome arm as the sex gene, it is not impossible that it is located in the other arm, where one would expect about 35% recombination. To detect such a percentage of recombination about twenty-two asci would need to be analysed before significant results could be expected.

In the course of the present study, the position of the sex gene, located much farther from the centromere than in *N. crassa*, has been confirmed, and three other genes on the sex chromosome have been mapped, with a view to testing for chromatid and chiasma interference.

#### MATERIAL AND METHODS

The stock of *Neurospora sitophila* used is that described by Ramsbottom & Stephens (1935) as having been found by W. H. Wilkins growing in enormous quantity on the ends of beech battens which were being kiln dried in a timber yard at Chichester in 1933. The + and - strains are morphologically indistinguishable, both producing abundant orange conidia. When 200 conidia from the + strain were isolated in separate tubes of agar and the mycelium allowed to grow to maturity, it was found that twenty-five were of a mutant type called *pink*, and when an ascus from a cross between the original + and - strains was dissected and the eight spores grown separately, it was found to have segregated for *pink* and *normal*, as well as for + and -. Three other mutants, *brown*, *orange* and *weak* appeared in single spore cultures of conidia treated with large doses of X-rays, and consequently were probably X-ray induced; several other abnormal forms were also obtained in the same way, but their characters are less well defined and they have not yet been analysed genetically. The chief characters subject to variation are the following:

- (i) The colour of the agar.
- (ii) The conidia (produced at the thinnest part of the agar slope).
- (iii) The non-conidial aerial hyphae (on the surface of the agar).
- (iv) The sclerotia, or false perithecia (usually on the glass).
- (v) The form and rate of growth of the hyphae.

The characteristics of normal *N. sitophila* and the four named mutants are given below, as they appear in mature cultures grown on Dox's agar at room temperature. On malt agar growth is more vigorous and the mutants are much less readily distinguished.

*Normal*. Agar with a slight yellowish tinge; conidia orange and abundant; aerial hyphae mostly clustered into rounded masses several millimetres in diameter; sclerotia abundant; growth extremely rapid, about 1.5 mm. per hr. at 27° C.

*Pink, Pk*. Agar a pink colour throughout, the colour being retained permanently; conidia pinkish orange, about one third as abundant as *normal*; aerial hyphae and sclerotia usually absent; growth very rapid but slightly slower than *normal*. There have been indications that *pink* survives both drying up of the agar and treatment by X-rays more readily than *normal*.

*Brown, B*. Agar uncoloured; conidia replaced by a dense mass of drops of brownish orange exudate (compare Lindegren's mutant *dirty*); aerial hyphae usually absent, but white fluffy mycelium occasionally appears in considerable abundance; sclerotia absent; growth very slow, one twentieth of *normal*, or less,

the hyphae tending to grow in circles. The cytoplasm of the hyphae seems to degenerate very readily. After the ascospores have germinated, for about a week at 27° C. the hyphae hardly grow at all, and out of 152 germinated spores that have been isolated, 94 died at this stage.

*Orange, O.* Agar coloured a bright orange at the surface, but the colour only lasts about two weeks; conidia about one-third as abundant as *normal*, borne on very short hyphae; aerial hyphae abundant, evenly distributed over the surface of the agar; sclerotia usually absent; growth slow, about one-fifth of the *normal* rate, with much more crowded hyphae.

*Weak, W.* Agar with a slight pink tinge; conidia weakly developed, about one-tenth as abundant as *normal*; aerial hyphae usually absent; sclerotia absent; growth very rapid but slightly slower than *normal* and with fewer hyphae.

The double mutants, *pink-brown* and *weak-brown* are similar to *brown* except that the brownish drops replacing the conidia are very much less abundant.

A screw micromanipulator is used for the dissection of asci, and the method finally adopted is as follows: Some perithecia are transferred to a drop of sterile water on a microscope slide by means of an inoculating wire, and crushed under a coverslip, so that the clusters of asci are ejected into the water. The perithecia must be of the right age; if too young the spores will be unripe, if too old the spores will have been shed from the asci. A cluster containing asci with all eight spores ripe is then picked up on the inoculating wire, under a prismatic binocular microscope, and transferred to Dox's agar on another microscope slide. The dissection of individual asci is done under another binocular microscope with  $\times 17$  eyepieces and a  $2/3$  in. objective, to give room for the fine glass needles of the micromanipulator between the objective and the slide. An ascus at the edge of the cluster is chosen and the needle inserted between spores 1 and 2, or between 2 and 3, numbering from the top end, and the tip of the ascus dragged away from the cluster. The ascus often stretches to three times its natural length before breaking. The point of breakage is extremely variable. The spores are then freed of the ascus wall and spaced at distances of about 1 mm. by keeping the needle fixed and moving the mechanical stage, thus keeping the spore and needle in the centre of the field. Occasionally an ascus pulls out whole at the base without breaking. A second needle is then usually necessary for dissection in order to anchor one end of the ascus. As each spore is isolated it is buried slightly in the agar to prevent drying up, which tends to inhibit germination, and its position is marked on a plan of the spores.

After dissection of several asci, the slide is placed on a frame of glass rods over water in a Petri dish, and the dish placed in an oven at about 55° C. for 1 hr. Twelve hours later in hot weather, 18–24 hr. in cold, the spores will have germinated and will be at a convenient stage for isolation. Each germinated spore is cut out with the inoculating wire, under the prismatic binocular microscope, and transferred to a slope of Dox's agar in a test-tube plugged with cotton-wool. Sex tests are best made the next day, before conidia have begun to be produced; this avoids opening tubes containing conidia, these being very light and powdery. A piece of agar carrying mycelium is cut out and transferred to a slope of malt agar, to which mycelium of known sex is added. Malt agar is used since perithecia do not form readily on Dox's agar. With cultures of *orange* one day must elapse and with *brown*

four or five days before adding the *normal* mycelium of known sex, to allow for the difference in growth rate. Lack of production of perithecia is not always a reliable test that two strains are of the same sex, since sterility is common.

The original stocks of the *normal N. sitophila* and of the various mutants showed a high degree of sterility. Perithecia were usually formed, but the majority contained no ripe spores at all, and to find an ascus with all eight spores ripe required a long search. By selecting the most fertile crosses, however, the fertility was rapidly increased in the course of one or two generations. In this connexion it is worth noting that the correlation between size and abundance of perithecia and fertility is very slight, large perithecia being often completely sterile and small often highly fertile and containing thousands of spores. The distribution of the perithecia is of interest; usually they are scattered throughout the + and - mycelia, but less frequently they are confined to the mycelium of one or other sex. For instance, a fertile stock of - *pink* has been obtained and when this is crossed with any strain of + *normal*, perithecia are always confined to the *pink* mycelium.

It is important that the asci dissected should have more than four of the spores ripe, because the paler coloured unripe spores will not germinate. Moreover, if the asci are showing segregation for slow and quick ripening of the spores and if, as is possible, spores at one end of the asci tend to ripen earlier than those at the other end, by dissecting asci with only four spores ripe, one may be unconsciously selecting asci that have segregated at the first division for the factor affecting ripening of the spores. Another practical point of importance concerns asci showing segregation for an inhibitor of germination, and it also applies to asci in which two pairs of spores have failed to ripen. If the inhibitor has segregated at the second division, first division segregation for other characters can never be detected, but it may be possible to detect second division segregation. For instance, supposing spores 3, 4, 7 and 8 have failed to germinate, if spores 1, 2, 5 and 6 are all of the same sex, then segregation for sex must have been at the second division; if, however, the two pairs that survived are of opposite sex, it is impossible to tell at which division segregation for sex took place. Hence, when counting the numbers of asci showing first and second division segregation for a pair of allelomorphs, asci which have segregated at the second division for an inhibitor of germination (or of ripening of the spores) must be omitted, whether the division required can be determined or not. If segregation for an inhibitor has occurred at the first division, the division at which segregation has occurred for other characters can always be determined, but if the inhibitor is linked to one or more of these characters, then by counting asci showing segregation for the inhibitor at the first division, but not those at the second division, there will be an unconscious selection for asci showing first division segregation for the characters linked to the germination-inhibitor.

#### RESULTS

The percentages of second division segregation obtained for the various genes are shown in Table 17. The figures in the second column give the total numbers of asci analysed and in the third column the numbers which showed segregation at the second division. The last column gives the uncorrected map distance of the



genes from the centromere, obtained by halving the percentage of second division segregation. Eighty-seven asci have been analysed for sex and 50 of these had segregated at the second division, or 57.5%, in good agreement with the figure of 54.2% obtained from the earlier data. *Pink* gives an even higher percentage of second division segregation, almost 70%, and *brown* and *weak* each give about

Table 17

Gene	Asci	2nd	% 2nd	Dist.
+	87	50	57.5	28.7
<i>Pk</i>	26	18	69.2	34.6
<i>B</i>	46	18	39.2	19.6
<i>W</i>	25	9	36.0	18.0
<i>O</i>	6	5	83.3	41.7

40%. Altogether, sixteen asci showing segregation for *orange* and *normal* were dissected, but eleven of them also showed segregation of a gene which caused the spores either to fail to germinate or to die young. This gene showed no linkage either to *orange* or to sex, but unfortunately ten of the eleven asci had segregated at the second division for the lethal, so that data on the division at which segregation for *orange* occurred are available for only six asci. They suggest that the gene for *orange* is far from the centromere of its chromosome.

Table 18

Genes	Half-asci			% Recomb.
	Total	Orig.	Recomb.	
<i>Pk</i> and +	53	42	11	21.2
<i>B</i> and +	92	80	12	13.0
<i>Pk</i> and <i>B</i>	30	22	8	26.7
<i>W</i> and +	56	34	22	39.3
<i>W</i> and <i>B</i>	46	31	15	32.6
<i>O</i> and +	24	12	12	50.0

The recombination obtained between the genes is shown in Table 18, in terms of half-asci. *Pink* and *brown* are linked to sex and, less closely, to each other, and *weak* is linked to *brown*, and, rather slightly, to sex. Taken in conjunction with the distances from the centromere, the disposition of these four genes is clear, and is shown in Fig. 6, *pink* being distal to the sex gene, *brown* proximal to it and *weak* in the other arm. All the distances are in good agreement. The figure of 34.6 for the distance of *Pk* from the centromere is not at variance with the other measurements, since distances from the centromere, when uncorrected for double cross-overs, are unlikely greatly to exceed 33.3. There is no indication that *orange* is sex-linked.

#### DISCUSSION

The fact that three of the four mutants tested should prove to be sex-linked, suggests that the number of chromosomes in *Neurospora sitophila* is small. The location of *pink* distal to the sex gene is of special interest, since the failure to find any genes in such a position in *N. crassa* led Lindegren (1936b) to suggest that sex

might be due to a distal differential region of the chromosome, where crossing-over was suppressed, instead of to a single gene.

The difference in the positions of the sex genes in *N. crassa* and in *N. sitophila* suggests that a translocation or an inversion of a segment of the sex-chromosome may have taken place during the evolutionary divergence of the two species. These two species are morphologically very similar and cannot be distinguished for certain except by examination of the asci (Shear & Dodge, 1927). They will hybridize and the hybrid is fertile. A study of segregation in the hybrid will be a method of approach to the elucidation of the differences in the chromosomes of the two species. In this connexion, Dodge (1931) has analysed twelve asci from a backcross between the hybrid and *N. crassa*; he found nine had segregated for sex at the first division and three at the second, giving 25% second division segregation.

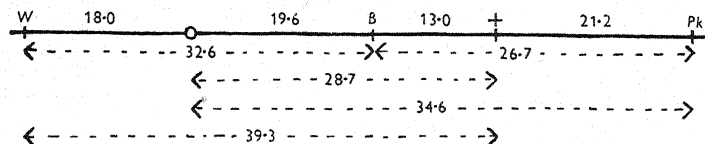


Fig. 6. Map of the sex chromosome of *Neurospora sitophila*.

The genes *weak*, *brown*, sex and *pink* are well placed for a study of chromatid interference, but *brown* is unsuitable since less than half the ascospores survive beyond the early stages of germination, and the double mutants *pink-brown* and *weak-brown* are probably indistinguishable.<sup>1</sup> It would be desirable, therefore, if possible, to find another gene, located close to *brown*, that could be used in place of it.

#### SUMMARY

1. Analysis of asci of *Neurospora sitophila* has confirmed that about 56% of asci show segregation for sex at the second division, compared with about 14% in *N. crassa*.
2. The gene for the mutant *pink*, obtained from the original stock, is located in the same arm of the sex chromosome as the sex gene, and distal to it. The genes for the mutants *brown* and *weak*, probably X-ray induced, are proximal to the sex gene, and in the other arm of the sex chromosome, respectively. A fourth mutant, *orange*, also probably X-ray induced, shows no linkage to sex.

This work has been made possible by a grant from the Department of Scientific and Industrial Research. I would like to express my thanks to Dr D. G. Catcheside for his helpful criticism throughout the course of the work.

<sup>1</sup> It has since been found that *brown* grows satisfactorily if yeast extract is added to the medium.

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## ON THE PRIMARY DISPERSAL AND ISOLATION OF FUNGAL SPORES

By C. G. DOBBS

*King's College, London*

(With 1 figure in the text)

### PART I. PASSIVE DISPERSAL

FUNGAL spores are dispersed through the two media, air and water. Both may at different times play a part in distributing the same spore, but it is probable that very few, if any, fungi are entirely restricted to one medium. It is convenient, however, to distinguish between *primary dispersal*, by which the spore is carried away from the parent body, and *secondary dispersal*, by which is meant any subsequent distribution after it has once come to rest.

Primary dispersal may occur within a one- or two-phase system formed by the two media (ignoring the phase constituted by the spores themselves), i.e. (1) in water alone, (2) in air alone, (3) in drops or on bubbles of one in a continuous phase of the other. Furthermore it may be *active*, the fungus supplying the initial energy, or *passive*, movement being due to the difference in density between the spore, or its containing drop, and the surrounding medium, together with movements of the medium or of other organisms, e.g. insects. With active spore dispersal, which has been the subject of extensive studies, I am not here concerned except to point out that it falls into the three classes mentioned above, as represented by (1) swarm-spores, (2) basidiospores, (3) ascospores. Biologically important as they are, most mycologists are in practice less concerned with these than with the numerous accessory spores produced in culture, which are all of the passively dispersed type.

Before the dispersal of these spores can be fully understood their behaviour both in water and air must be studied. The movement of spores through water can be observed under the microscope, whereas in general their movement through air cannot. This probably accounts for the neglect of the study of passive air dispersal as compared with water dispersal and active spore- or spore-drop-shooting, in which there is often a conspicuous discharge mechanism, and the sudden disappearance of the spore can be seen and its trajectory determined.

Since passively dispersed spores are difficult to find, the simplest method available for the study of this type of air dispersal is to receive them on a nutrient medium on which they will develop into colonies which can readily be seen.

The information incorporated below has been obtained by use of the simple 'blower' described in Part II, and also by spore-shedding tests in which the fungus, grown in a flask on the expanded end of a glass tube, is free to shed its spores on to a nutrient medium in the bottom.

If the spore-shedding capacity of the fungus is tested while it is moist and dry, in still air, and subjected to gentle and fairly strong 'puffs' from a rubber bulb



(avoiding violent blasts such as will detach whole spore masses or pieces of mycelium), it is possible to carry out a preliminary classification with respect to air dispersal as follows:

*Spore-retainers.* Shed no spores (or very few spores or spore masses over a long period when dry) under any of the above circumstances.

*Spore-drop-shedders.* Spore retainers in general, but scatter spore-containing drops when moist and subjected to rather strong blowing or shaking of their support.

*Dried-spore-shedders.* Shed few or no spores while moist, but broadcast many when dry and subjected to gentle blowing.

*Spore-shedders.* Shed some spores in still air soon after they are produced on the moist colony. Broadcast many spores when subjected to gentle blowing.

If spore-shedding tests are carried on long enough some idea can be gained of the length of the period of primary air dispersal. Sporangiophores of *Rhizopus nigricans*, for instance, which were first observed to drop spores in March 1939 were found two years later to be still rigid and erect and capable of shedding a few viable spores when subjected to tapping of their support.

Such tests, however, give information about air dispersal only and need supplementing by direct observation with the microscope on moist and dry cultures of the fungus. Owing to its relative density and power of dissolving mucilages water is capable of dispersing all fungal spores, but there is need for an investigation on the clumping and 'wettability' of spores in water. General observation shows that fungi may be dispersed solely by water, they may occasionally be carried through water on the surface of bubbles, or they may be *spore-drop-formers*.

An attempt to combine what is known about dispersal in both media in a tentative grouping of the main types of passive primary spore dispersal is given below, in order of increasing importance of air and decreasing importance of water. It is based upon general observations on the fungi during the past eight years, with spore-shedding tests carried on intermittently since 1938, and will no doubt need modifying when a greater bulk of precise information becomes available. The word 'spore' should be interpreted in the broadest sense.

Clearly a given spore type may be dispersed in several different ways, but may nevertheless be classified in one of the following groups according to its predominant method of dispersal.

#### *Groups I-III. Continuous phase, water.*

I. Single phase: primary dispersal by water (with varying amounts of secondary dispersal by air).

(a) Spores produced in and separated by external water (i.e. water not extruded by the fungus), e.g. oidia of many fungi in nutrient solutions.

(b) Spores produced in pools of water or slime extruded by the fungus, and dispersed by natural water (rain, dew, soil water), e.g. *Fusarium* spp., probably most Melanconiales.

The majority of these are *spore-retainers*, the spores being stuck together by mucilage when they dry down in situ. This prevents primary air dispersal, though not secondary air dispersal after they have been separated by water. Some primary dried-spore-shedding may occur, but is subsidiary to water dispersal.

II. Two phases: gas in water. Spores (oidia) carried upwards on bubbles as in 'working' yeasts. If we interpret the yeast colony rather than the cell, as the 'parent body' this must cause a certain amount of primary dispersal.

III-V. Two phases: water in air. *Spore-drop-formers*—spores, while on the parent mycelium, enclosed for a time in 'spore-drops'.

III. Primary dispersal by water. Spore-drops may run together into larger drops or pools (difficult-walled mucors) or into slimy masses (pyncospores of coelomycetes), and the spores are dispersed by natural water as in group I (b). Where the drops run together very early and new spores are formed in them this group merges into I (b). Where the drops do not run together they may dry down and form spore masses on the mycelium, or on solid objects which they happen to touch. The spores in these are usually firmly stuck together, but are readily dispersed by natural water (e.g. probably most soil mucors). Where the spore-bearing hypha is long, one may say that its elongation causes a certain amount of 'active' spore dispersal through air, e.g. *Phycomyces*, *Pilaira*.

*Spore-retainers*. As in group I, some primary dried-spore-shedding may occur, but is subsidiary to water dispersal. In some fungi, however, the two media seem to be used to a comparable extent, e.g. *Mucor racemosus*. Secondary air dispersal also occurs, e.g. of mucors on soil dust.

#### Groups IV-VI. Continuous phase, air.

Secondary water dispersal is always possible, and is predominant in group IV.

IV. Spore drops distributed through air,

(a) By insects or other animals or solid objects (oidia of *Coprinus*, rust pyncospores, probably many mucors and other fungi which grow on dung or organic remains).

(b) By fairly strong air currents or shaking of their support. *Spore-drop-shedders*. Peronosporales, some Mucorales, e.g. *Dicranophora*, probably many fungi which form drops on tall erect sporophores.

V. Spore drops drying down in situ, some dry spores being dispersed by air. *Dried-spore-shedders*. *Rhizopus*, *Absidia*, some mucors with fragmenting walls, e.g. *Mucor racemosus*. Judging by preliminary tests, aerial mycelium of some *Fusaria* and several soil Hyphomycetes not fully identified.

VI. Single phase, air. Spores not enclosed in drops, often not wetted when drops are present. Dispersed directly by air. *Spore-shedders*, by far the largest group. Many, but not all, 'conidial' Mucorales, e.g. *Piptocephalis*, *Syncephalastrum*, *Cunninghamella*. Most common Hyphomycetes, e.g. *Penicillium*, *Aspergillus*, *Monilia*, *Botrytis*, *Cladosporium*, *Alternaria*. Also many spore types not produced in culture, e.g. of smuts, rusts and puff-palls.

The work started with the Mucorales in which some mucors have been shown to be spore-drop-formers (see Dobbs, 1939). Spore-shedding tests then showed that the thin-walled mucors (*Mucor hiemalis*, *M. rouxianus*) are spore-retainers, as also are *Zygorhynchus* and *Pilaira*.

*Rhizopus* [see also Ingold (1940), who noted its air dispersal], *Absidia*, and to some extent *Mucor racemosus* and other unidentified mucors with fragmenting

walls, were shown to be dried-spore-shedders, and several conidial forms, *Piptocephalis*, *Syncephalastrum*, *Cunninghamella*, to be spore-shedders.

The tests were soon applied to other groups of fungi, particularly the Hyphomycetes, which were shown to fall into the same general groups before my attention was drawn to the morphological distinction between slime-spores and dry-spores made by Mason (1937), in the new classification of Hyphomycetes recently brought out by Wakefield & Bisby (1941). This distinction, which is the basis of their main division into Gloiosporae and Xerosporae, is concerned, not with the dispersal of the spores, but with the way they are 'presented for dispersal' and are freed from their points of attachment to the parent hyphae.

Approaching the matter independently from the physiological side, and working chiefly on a group in which this distinction does not precisely apply, I had arrived at the somewhat different conception of 'primary dispersal', which is concerned with the separation of the spore, not only from its point of attachment but from the plant on which it grows, and its dispersal to a place at which it might give rise to a new plant.

Nevertheless, as would be expected, the physiological groups correspond broadly to the morphological ones. Group VI (spore-shedders) will probably be found to include all the Xerosporae, and the Gloiosporae will be divided up among groups I-V, in what proportion remains to be seen.

Mention should also be made of the work of Stepanoff (1935), to which Mason's paper has drawn my attention. Confining himself to parasitic fungi, he tested their dissemination by air currents and distinguished three groups: (1) very easily detached and carried away (corresponding to my spore-shedders), (2) detached and carried away with difficulty (probably include spore-drop-shedders and some I have regarded as spore-retainers), (3) not disseminated by air at all (spore-retainers).

The first two groups, which include the majority of fungi tested, are classed as 'anemochores', the third is divided into 'hydrochores' and 'zoochores', and mention is made of 'other mechanical means' which may include some 'active' spore dispersal. The physiological study of the movement of spores in air, which I have not attempted here, is carried out in detail, but (judging by the abstract which alone is available) the distinction between active and passive, primary and secondary spore dispersal appears not to have been made.

As I have been working chiefly on saprophytic moulds, and Stepanoff on plant parasites, the two studies are largely complementary.

These conclusions are published at this preliminary stage in the hope that they will be stimulating and useful, and because it may be long before a fuller treatment can be attempted, but they are in no sense intended to be final.

## PART II. ISOLATION OF AIR-BORNE SPORES

It is a remarkable fact that, whereas a large proportion of the fungal spores produced in culture are naturally air-borne—Wakefield & Bisby place three-quarters of the British Hyphomycetes in the Xerosporae—yet water plays the chief part in all the methods of spore isolation in common use.

In the comprehensive summary of techniques for the isolation of single micro-organisms given by Hildebrand (1938), without exception all the methods described involve *primary* dispersal of the spores or bacteria in a watery liquid. Secondary dispersal may involve the use of droplets (corresponding to group III) or the so-called dry-needle method which picks up a slimy spore from a wet surface (corresponding to group IV (a)), but there is no instance of the use of the natural medium for the dispersal of dry spores.

Water dilution is the only available method for many water-dispersed spores, which either will not separate, or sometimes will not survive in air. It can also be used for air-dispersed spores, all of which can exist for a time in water which is

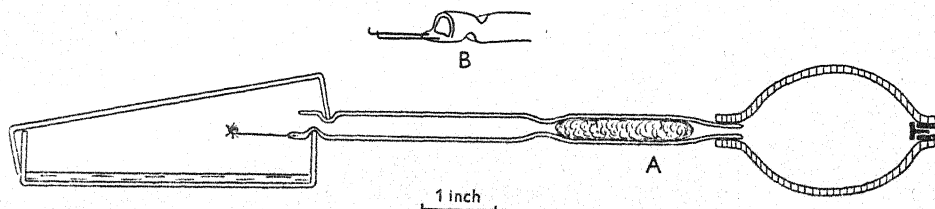


Fig. 1. A, Diagrammatic section of blower in use. B, Oblique view of mouth of blower.  
For description see text.

essential for their germination; but it is not necessarily the best method. Dry spores of *Rhizopus*, for instance, do not wet readily, and hang together in festoons on the surface of the water, whereas they separate freely in air.

As it seemed reasonable to suppose that an isolation method which corresponds closely to the natural means of dispersal of the spores would possess advantages, an investigation was started into the technique of blowing dry spores on to a wet medium in which they could germinate, which is roughly what occurs in nature.

A simple insufflator (or more shortly, blower) was therefore devised for blowing dry spores off the parent mycelium on to nutrient medium in a Petri dish, and has been found useful and effective. It consists (see Fig. 1) of a pyrex glass tube having a rubber bulb with inlet valve at one end, and two prongs of tungsten wire, fused in just below and projecting in front of the mouth. At a point nearer the bulb the tube is drawn into a slight waist above which it is plugged with cotton-wool.

The function of the plug is not only to filter the air, but also to damp down the velocity of the blowing, which can be varied by adjusting the length and density of the plug, the size of the exit hole and the distance of the wire hook from it, as well as the rate of compression of the bulb.

The part of the blower below the plug is flamed, and a tuft of aerial mycelium is picked up on the prongs, or if necessary a piece of the culture medium can be cut out and placed on them. A third method is to take up a very small wisp of sterile cotton-wool on the wire and touch that on the sporing surface, but with very fine-



spored species, e.g. of *Penicillium*, all that is necessary is to touch the surface with the wire itself, as when inoculating normally. The end is then pushed under the lid of a Petri dish, the edges of which are closed on to the 'kinks' behind the mouth of the blower, as shown in the diagram. One gentle puff is usually all that is needed. The procedure is then as with an ordinary dilution plate or loop streak.

If the blowing has been judged rightly well-spaced colonies appear on the dish, and in the early stages many of them can be identified as arising from a single spore, and can easily be picked up with a scalpel or dummy objective. Too violent blowing results not only in crowding but in a higher proportion of multispore colonies. Where, however, the mycelium is moist, since drop dispersal is not wanted, the blower may be used hot with advantage.

As an example of the effect of this method, a single puff on a small tuft of *Rhizopus nigricans* gave 128 colonies, with the following distribution as to spore numbers:

numbers:																		about		
No. of spores	1	2	3	4	5	6	7	8	9	10	12	13	15	16	18	22	40	80	500	
Colonies	71	18	7	5	4	2	4	3	3	1	1	2	1	1	1	1	1	1	1	

With fine-spored *Penicillia* the same treatment gives several thousand colonies on the plate, and a scarcely perceptible puff is required to give an optimum distribution.

For spore isolations the chief advantage of this method over those in common use is its extreme simplicity, operations being reduced to an absolute minimum, so that if necessary it can be repeated with greater frequency; only the surface of the medium need be examined for spores, whereas in a dilution the whole thickness must be searched, and even in a 'streak' the wire sometimes digs spores into the medium.

It has also certain other uses, for example:

(1) It provides a simple practical test for distinguishing a spore-retainer from a spore-shedder. If no colonies appear after gentle blowing, and if violent blowing detaches only a few spore-masses, or pieces of mycelium, a water-dispersal method for isolation is indicated, but where many colonies appear after the first blowing, their number gives an indication of the effectiveness of the spore type in air distribution.

(2) It is the simplest means of separating spore-shedding fungi from spore-retaining ones (e.g. *Rhizopus* or *Absidia* from *Mucor*), or from moist bacterial colonies.

(3) It is also effective in separating out a mixture of spore-shedding fungi, and may reveal the presence of previously unsuspected strains or species, provided that they are not spore-retainers. Dried bacteria on the hyphae are also separated out on the plate.

(4) It is useful in teaching for inoculating class material, especially where slow-growing species are required to cover the plate quickly, or it is desirable to give each student one or more whole colonies to examine, and can be used for mixing the colonies of two strains on the plate, either to avoid staling effects, to get sexual reactions all over the plate, or for infecting with a parasite (e.g. *Piptocephalis*).

The main objection to the method seems to lie in the possibility of infecting the laboratory with spores, and for that reason it is best not done in the culture room. The chief danger arises not during the blowing, which is done inside a nearly closed Petri dish, the spores being blown away from the gap, but while the sporing hyphae are exposed to the air on the hooks of the blower. These should, of course, be flamed immediately after the operation, and if the exposure is reduced to a minimum, and the blower is handled gently in still air, the infection caused need be no more than with an ordinary inoculating needle.

#### SUMMARY

*Part I.* A distinction is drawn between active and passive, primary and secondary, spore dispersal, and a tentative grouping of passive primary spore dispersal types is outlined, in order of increasing importance of air and decreasing importance of water. A majority of fungi tested in air are found to be *spore-shedders*, a minority *spore-retainers*.

*Part II.* It is pointed out that hitherto all methods of spore isolation have made use of water as the primary dispersal medium, and a simple method of isolation by air is described.

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## CYTOLOGICAL AND ECOLOGICAL NOTES ON SOME SPECIES OF *GALIUM* L. EM. SCOP.

By B. L. HANCOCK

*Department of Botany, Oxford*

(With 2 figures in the text)

THIS work began, at the suggestion of Dr A. R. Clapham, with a cytological investigation of forms of *Galium palustre* L. which grow in the neighbourhood of Oxford. Later it was extended to *G. uliginosum* L., to material of *G. palustre* from a number of other localities, and to *G. debile* Desv. from Chudleigh Knighton, Devon. Since the work was begun in the late autumn of 1940 when flowering and fruiting had been completed, and since only one academic year was available, cytological work was commenced on material whose accurate identification was impossible at the time. All plants whose chromosome number was counted were transferred to pots for subsequent examination. These plants are still available (January 1942).

### CYTOLOGICAL TECHNIQUE

Somatic chromosome counts were made from actively growing root tips. These were conveniently obtained by placing the plants in water which was changed daily to improve aeration. An abundant supply of relatively thick and straight roots, mostly adventitious, was produced in a week, although in winter the supply was less certain.

Excellent fixation was obtained with Belling's Navashin type fixative. Owing to their small diameter, the root tips were mounted together on cards (Randolf, 1940) before embedding in paraffin wax of m.p. about 50° C. Sections were cut transversely at 8 or 10  $\mu$  for the higher polyploids, with a Spencer rotary microtome. The chromosomes were stained with crystal violet by Randolph's method (quoted by Newcomer, 1938) which includes mordanting in 1% chromic acid for 20 min. before staining. The best results were obtained by staining for 2½ hr. in 0.1% crystal violet, increasing the time in alcoholic iodine to 5 min. and shortening the differentiation to about 45 sec. in absolute alcohol and about 12 sec. in clove oil, but adding about 1 min. of slow differentiation in a mixture of 2 parts xylol to 1 part absolute alcohol, after 2 min. in xylol. The following is the complete schedule (for further details see Hancock, 1942):

- |  |           |
|--|-----------|
| 1. Fix the root tips in Belling's Navashin type fixative. Evacuate for a few minutes and leave | Overnight |
| 2. Harden in 50% alcohol   | 3 hr.     |
| 3. Mount root tips on card   |           |
| 4. Dehydrate and harden gum in 70% alcohol   | Overnight |
| 5. Continue dehydration and embedding in paraffin wax by the chloroform method (La Cour, 1937) |           |

- |  |                        |
|--|------------------------|
| 6. Cut sections (transversely for chromosome counts) about $10\mu$ in thickness                        |                        |
| 7. Dissolve out the wax in xylol and take the slides down from ethyl alcohol to water in the usual way |                        |
| 8. Mordant in 1 % aqueous chromic acid   | 20 min.                |
| 9. Rinse in tap water and then in two or three changes of distilled water                              | About 10 min.          |
| 10. Stain in 0.1 % (or 0.5 %) aqueous crystal violet   | $2\frac{1}{2}$ hr.     |
| 11. Rinse in tap water   |                        |
| 12. Iodine and potassium iodide in 80 % alcohol  | 5 min.                 |
| 13. 95 % alcohol   | 2-3 sec.               |
| 14. Three changes of absolute alcohol  | A total of 30-45 sec.  |
| 15. Clove oil  | 10-15 sec.             |
| 16. Xylol  | 2 min.                 |
| 17. Xylol 2 parts : absolute alcohol 1 part  | 45-100 sec.            |
| 18. Three changes of xylol   | A total of about 2 hr. |
| 19. Mount in canada balsam   |                        |

The chromosomes were counted with a Zeiss binocular microscope, using a Zeiss apochromatic 1.5 mm. objective together with compensating  $15\times$  eyepieces, giving a magnification of *c.* 700 diameters.

#### CYTOLOGICAL RESULTS

*Galium palustre* L., in the Oxford district, consists of at least two races; a diploid ( $2n=24$ ) and an octoploid ( $2n=c. 96$ ). In Devon, a tetraploid ( $2n=48$ ), which has not previously been reported, was found; and, in the same locality, *G. debile* Desv. was found to be another diploid ( $2n=24$ ). *G. uliginosum* was found to have 22 somatic chromosomes.

The chromosomes are very small, usually between 1 and  $2\mu$  in length, and 0.3 and  $0.4\mu$  in diameter; no appreciable differences could be detected (Fig. 1). To what extent the slight differences in swelling are due to differences between the plants or to the fixation and subsequent treatment, is uncertain, but since most of the range of variation is shown within a single population it does not appear to reflect any important differences.

No polysomy could be confirmed, but it may be present in the octoploid *G. palustre*. It was found impossible to count the chromosomes of this plant with complete accuracy. There were always a few groups of chromosomes whose interpretation was in doubt, so that a plant might have one or two chromosomes more or less than 96 without this being detected. That most had 96 chromosomes is suggested by the clustering of all the more accurate counts round 96; half of the total number of counts differing by two or less, a quarter by one or less, and one-sixth, including the most accurate count, being exactly 96. However, there were a few fairly accurate counts which differed by about four, suggesting that these plants may exhibit polysomy; and where the somatic number is high it is unlikely that a few chromosomes more or less will seriously affect the balance. In addition, the octoploid in particular reproduces vegetatively by means of creeping shoots which root at the nodes.

A very few observations of meiosis in acetocarmine smears suggest that, even in the octoploid, meiosis is extraordinarily regular without frequent multivalent formation.



THE DIFFERENTIATION OF POLYPLOID TYPES IN *GALIUM PALUSTRE* L.

*Galium palustre* is a common plant in the Oxford district where it occupies a fairly wide range of damp habitats, but it is almost entirely restricted to marsh habitats on inorganic soil with relatively good aeration, such as is the result of a

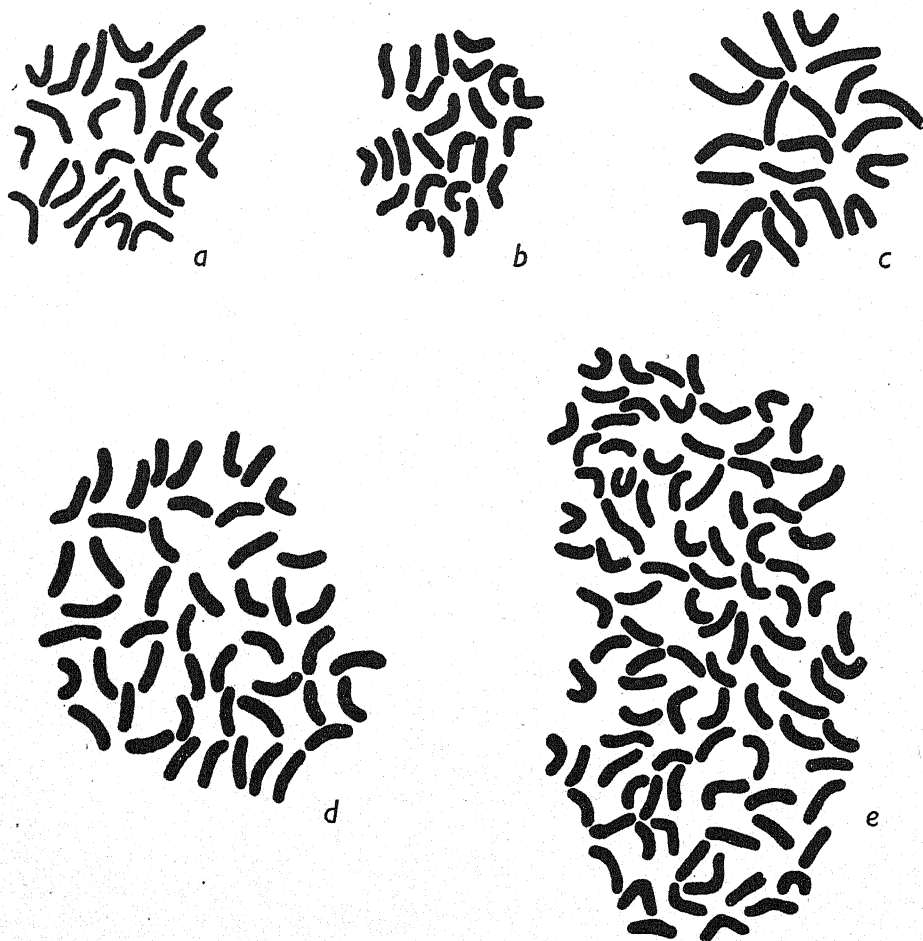


Fig. 1. Metaphase plates from mitoses in root-tips. (a) *Galium palustre* L., diploid  $2n=24$ .  $\times c. 4600$ . (b) *Galium debile* Desv., diploid  $2n=24$ .  $\times c. 5000$ . (c) *Galium uliginosum* L., diploid  $2n=22$ .  $\times c. 5000$ . (d) *Galium palustre* L., tetraploid  $2n=48$ .  $\times c. 5700$ . (e) *Galium palustre* L., octoploid  $2n=96$ .  $\times c. 4900$ .

varying water-table. These habitats can be divided into two kinds. One is submerged most of the winter and during the summer has the water-table very close to the surface, usually due to the proximity of a pool or a stream. It is always very damp, but an oxidizing potential is preserved by some movement of water. The other is also submerged during the wetter period of the winter when the plant is least influenced, but during the summer it becomes much drier. This kind of habitat is typically seen in the meadows surrounding the Thames near Oxford.

This division of ecological preferences corresponds with the cytological division. The diploid grows in the damp places which dry out in summer, while the octoploid is found in the permanently damp zones, often bordering upon water. This difference is shown in the appendix, where the habitats of the plants counted are outlined. Fagerlind (1937, p. 356) confirms the ecological difference between the diploid and the octoploid; he saw the draining of a lake in Sweden where the octoploid was abundant in a zone of *Phragmites* and the diploid was growing on the drier ground behind. In the following year, the open water was drained and the diploid was abundant, but he was unable to find any octoploid.

In the Oxford district, however, ecological difference is not absolute. In one community, which grows in a slight depression just west of Hagley Pool, both the diploid and the octoploid are abundant. This is probably slightly damper than the typical diploid habitat, but is much drier in summer than is usual for the octoploid, which must be rather more tolerant in its ecological requirements than the diploid.

The tetraploid has not yet been found near Oxford but was growing in Devon in an intermediate habitat, often submerged in winter and damp in summer, but without having the water-table constantly near the surface. The greater ecological tolerance of the octoploid is again shown, since one of the plants from this population has been identified, on morphological grounds, and by its stomatal size, as the octoploid.

There appear to be no clear qualitative morphological differences between the polyploid races, suggesting that they are autopolyploids, but *gigas* characters are shown. The diploid is, in the Oxford district, a small plant with small leaves, flowers and fruit, flowering about a fortnight earlier than the octoploid. The latter is a much larger and more robust plant with thick stems and much larger leaves and flowers. In the mixed population near Hagley Pool the mature plants were readily separated into two groups with a very few intermediates. Measurements of stomatal size (see below) confirmed this separation and enabled the intermediates, which on closer examination appeared to be either extra large diploids or rather weak octoploids, to be identified. The tetraploid is intermediate in these *gigas* characters, including stomatal size. Another difference between the polyploid races is the diameter of the rootlets when growing in water or very wet soil. The diploid has very slender rootlets, the tetraploid moderately thick, and the octoploid comparatively stout rootlets. This is useful in distinguishing the races in early spring, and was used to detect cytological heterogeneity in a population, by collecting the most extreme types, growing them in water, and counting those with the thinnest and the thickest rootlets. In this way it was possible to detect mixed populations by counting comparatively few plants.

In spite of these quantitative differences, there was some overlapping in the range of variation, weak octoploids being smaller than strong diploids and the intermediate tetraploids may overlap with both. However, it was found to be relatively easy to distinguish between the mature plants growing naturally in the Oxford district and in some localities in Devon.

Stomatal size is a relatively easily determined character which appears to provide a fairly accurate means of identifying the types. It was determined by measuring the length of the guard cells in pieces of the lower epidermis, stripped from the

middle of the leaf just to one side of the midrib and mounted in water. Mature, fully expanded leaves were used. The results are shown in Table 1.

Table 1. *Lengths of guard cells of stomata in  $\mu$ . Each figure is a mean of twelve measurements from a single plant whose chromosome number has been counted or which comes from a population sampled for chromosome counts*

	Fresh material from natural habitat	Mean	Fresh material grown in pots	Mean	Dried material soaked out for examination
Diploid	30.0, 27.5, 28.7, 27.4, 30.3, 28.2, 30.2, 27.9, 28.7	$28.8 \pm 0.38$	25.2, 25.1	25.2	—
Tetraploid	—	—	30.4, 32.4	31.4	28.9
Octoploid	40.9, 38.1, 41.2, 35.9, 38.2, 41.3, 37.9, 37.5	$38.9 \pm 0.71$	37.7, 36.2 35.5, 34.4	36.0	37.6

It will be seen that diploids and octoploids can be distinguished sharply by this means. Measurements for tetraploids were made only on pot-grown or dried material. They showed no overlapping of range with comparable diploids and octoploids, but the data are very scanty and it cannot yet be stated that tetraploids can be identified with certainty by this technique.

*Galium debile* Desv. is very closely related to *G. palustre* and has been treated as a subspecies (Rouy, 1903). It is a diploid plant with the same number of chromosomes as the diploid *G. palustre*. This suggests that it is a species which does not hybridize with *G. palustre* in nature, although it is true that the diploid *G. palustre* does not grow near its Devon locality and, at Hatchett's Pool in the New Forest, the associated *G. palustre* has not been identified. However, *G. debile* is a distinct plant in its morphology and probably in its ecology. At Hatchett's Pool *G. debile* has been described as occupying the same damp and rather acid (judging by the associated plants) zone as the unidentified *G. palustre*, but, in its Devon locality, there is no overlapping of *G. debile* with the zone of the tetraploid *G. palustre* (together with a little octoploid) which grows among *Molinia* tussocks, although in places they are less than a metre apart. *G. debile* grows in a pool completely submerged in winter and spring and is especially abundant in the shallower parts of the pool. But in summer the pool disappears and *G. debile* grows in the dry bottom. This habitat resembles that of the diploid *G. palustre* but is wetter for a much longer period and may be drier in summer.

In morphology *G. debile* is even more distinct from *G. palustre*. When in flower it is a small plant, very similar to the diploid *G. palustre* except that it has small, linear and almost succulent leaves. However, in spring, when the shoots are submerged, *G. debile* bears long, thin, linear leaves 2-4 times as long as the aerial leaves (Fig. 2). These water leaves are so long and thin that they collapse together when taken from the water. This extraordinary water form, which is described by Glück (1911), is quite different from anything found in *G. palustre* whose submerged leaves are merely somewhat smaller and thinner than those borne later.



Fig. 2. A drawing of shoots of *Galium debile* Desv. found submerged in the pond at Chudleigh Knighton, Devon, on 3 April 1941.



*G. debile* appears to be a distinct species which has diverged from the *G. palustre* stock without any change of chromosome number.

*Galium uliginosum* L. differs from the preceding species in being a fen plant, confined, in the Oxford district, to base-rich habitats which are permanently moist at the surface, though with little or no standing water in the summer. Such habitats are provided in the calcareous peat of the small fens at Cothill, Headington Wick, etc., and, to a smaller extent, in permanently wet depressions of the alluvial meadows by the Thames and on Otmoor. Morphologically, also, it is a relatively constant species differing from *G. palustre* in having more leaves in each whorl, more pronounced, recurved prickles and terminal mucros to the leaves, and with the flowers in small axillary panicles, unlike the large terminal panicle of *G. palustre* and *G. debile*. *G. uliginosum* is like *G. debile* and unlike *G. palustre* in that it does not turn black on drying. It seems likely that, since 11 is the more common basic number in the genus (Fagerlind, 1937), *G. palustre* evolved from *G. uliginosum* as a tetrasomic and *G. debile* separated from *G. palustre* before the latter acquired its character of turning black on drying and, subsequently, two other types have been produced from *G. palustre* by autopolyploidy.

#### SUMMARY

Cytological, ecological and morphological investigations on the marsh and fen *Galium* spp. of the Oxford district and of a Devon locality show that *Galium palustre* L. consists of three polyploid races; the diploid and octoploid with distinct ecological preferences in the Oxford district, and the tetraploid found, with some octoploid, in Devon. *G. debile* Desv. is a closely related diploid growing in Devon in a rather similar, but more extreme, habitat to the diploid *G. palustre*, drier in summer and submerged longer in winter and spring when it exhibits its extraordinary water form with very long linear and thin leaves. *G. uliginosum* L. differs in being a fen, not a marsh, plant in the Oxford district and it has 22 chromosomes, not 24 like the diploid *G. palustre*. The differences between the polyploid races of *G. palustre* are considered; the most striking morphological differences are the *gigas* complex of characters associated with polyploidy, including stomatal size, which appears to provide a fairly accurate means of identification.

I am deeply indebted to Dr A. R. Clapham, at whose suggestion the work was commenced, for constant help and encouragement.

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## APPENDIX

*Details of communities from which chromosome counts were made*

Designation of plant	Habitat notes	Somatic number of chromosomes
<i>Galium palustre</i> L.	Wytham, Berkshire	
2, 4, 7, 9, 110, 114 (6 plants)	In a zone bordering Hagley Pool among marsh plants including <i>Sparganium ramosum</i> , <i>Carex riparia</i> , <i>C. acuta</i> , <i>Oenanthe fistulosa</i> , <i>Sium latifolium</i> , <i>Menyanthes trifoliata</i> , <i>Eleocharis palustris</i> , <i>Equisetum limosum</i> , <i>Myosotis palustris</i> , <i>Mentha aquatica</i> and <i>Hypnum cuspidatum</i>	c. 96
31, 32, 33, 39, 42, 43 (6 plants)	In an area of the alluvial meadows which is periodically submerged during the winter but drier in summer, just west of Hagley Pool. Associated plants include <i>Festuca rubra</i> , <i>Juncus articulatus</i> , <i>Ranunculus repens</i> , <i>Carex panicea</i> , <i>Orchis incarnata</i>	24
23, 24, 26, 28 (4 plants)	The same community as the above (plants 31-43)	c. 96
North of Wolvercote and West of the Woodstock Road, Oxfordshire		
59, 62, 66, 70, 76 (5 plants)	In an alluvial meadow wet in winter but drier in summer, like the previous community, with <i>Juncus inflexus</i> (d.), <i>Agrostis stolonifera</i> (a.-co.d.), <i>Ranunculus repens</i> (a.), <i>Trifolium repens</i> (a.), <i>Hypnum cuspidatum</i> (l.a.), etc.	24
Greenham Common, Berkshire		
95 (1 plant)	At the head of a small valley in the heath and often submerged in winter but drier in summer. Associated plants include <i>Juncus acutiflorus</i> , <i>Agrostis stolonifera</i> , <i>Ranunculus Flammula</i> , <i>Myosotis palustris</i> , <i>Epilobium palustre</i> and <i>Hydrocotyle vulgaris</i>	24
100, 102 (2 plants)	At the head of a similar valley 200 yards south, with similar associated plants	24
Cothill, Berkshire		
84, 85, 88, 90 (4 plants)	In and on the bank of a shallow drain crossing Morland's Meadow which carries water after rain. These plants may be relics from the time when the drain was deeper and regularly carried water. Associated plants include <i>Juncus subnodulosus</i> , <i>Molinia coerulea</i> , <i>Filipendula Ulmaria</i> and <i>Scabiosa Succisa</i>	c. 96
Near Padworth, Berkshire		
104, 105, 106 (3 plants)	In a deep channel in an alluvial meadow just south of the canal with <i>Juncus inflexus</i> , <i>Epilobium hirsutum</i> , <i>Ranunculus repens</i> , etc. In late March the plants were submerged	c. 96
Near Chudleigh Knighton, Devonshire		
118, 122 (2 plants)	In a damp zone round an old clay pool among <i>Molinia coerulea</i> tussocks together with <i>Juncus acutiflorus</i> (a.), <i>Juncus effusus</i> (l.a.-d.), <i>Mentha aquatica</i> , <i>Hydrocotyle vulgaris</i> , <i>Lotus uliginosus</i> , etc. A fairly accurate count on a third plant gave $2n=47$	48

Designation of plant	Habitat notes	Somatic number of chromosomes
<i>Galium debile</i> Desv.	Near Chudleigh Knighton, Devonshire	
123, 125 (2 plants)	In the claypool bordered by <i>G. palustre</i> . The plant is submerged in winter and spring but the pool dried out almost completely in summer. Associated plants include <i>Scirpus fluitans</i> (a.-d.), <i>Juncus bulbosus</i> (a.-l.d.), <i>Ranunculus Flammula</i> (a.), <i>Glyceria fluitans</i> , <i>Agrostis stolonifera</i> , <i>Veronica scutellata</i> , <i>Hydrocotyle vulgaris</i> , <i>Hypnum exannulatum</i> .	24
<i>Galium uliginosum</i> L.	Wytham, Berkshire	
14, 17, 21 (3 plants)	A 'hanging fen' where base-rich water seeps out south-west of Hagley Pool with <i>Carex riparia</i> , <i>Juncus inflexus</i> , <i>J. articulatus</i> , <i>J. acutiflorus</i> , <i>Eriophorum angustifolium</i> , <i>Vicia Cracca</i> , <i>Orchis Fuchsii</i> , <i>O. praetermissa</i> , etc.	22
	Cothill, Berkshire	
78, 79 (2 plants)	A permanently wet part of the fen in Parsonage Moor with <i>Schoenus nigricans</i> and <i>Juncus subnodulosus</i> locally dominant	22

## REVIEWS

*A Revision of Melanconis, Pseudovalsa, Prosthecium and Titania.* By LEWIS E. WEHMEYER. 9 $\frac{1}{4}$  × 6 in. Pp. viii + 161, with 11 plates. Ann Arbor, Michigan, U.S.A.: University of Michigan Press. 1941. Price 2.50 dollars.

Dr Wehmeyer is engaged in revising the stromatic Pyrenomycetes, but especially the genera grouped by other authors in a proposed family Diaporthaceae, that he has not yet himself accepted. This monograph may be considered an addendum to his *The genus Diaporthe Nitschke and its segregates*, published in 1933. The species of the present genera inhabit the bark of broad-leaved European and North American trees; but, unlike most species of *Diaporthe*, each is usually confined to one host genus, and usually also to the dominant species of that genus. Once again European and Pacific coast species are found to resemble one another more nearly than they do their eastern American counterparts.

In general appearance these fungi resemble those species of *Diaporthe* that develop their perithecia in discrete aggregates, but differ in not forming a black line either on the surface of the host or on that of each perithecial aggregate. In structure, their perithecia resemble those of *Diaporthe* and of genuine *Valsa* in the major character that their asci, at first interspersed with broad paraphyses, finally become free and completely fill the lumen of each perithecium. Each ascus tip usually is uniformly thickened and hence does not display the refractive ring that is such a feature in *Diaporthe*.

In this group, the stromata vary from hyaline and superficial to fuscous and immersed; and the ascospores from one-septate, hyaline, without appendages to six-septate, brown, with appendages. The conidia when present often occur as A- and B-spores; the A-spores vary from o-septate, hyaline, superficial to seven-septate, brown, immersed.

In the thirty-five accepted species, however, development has not progressed *pari passu* in stroma, in ascospore, and in conidium; and so the author has been compelled to choose between accepting either a few broad, and arbitrarily limited genera or numerous monotypic, vaguely defined ones. Believing that classification should not only reflect affinity but also place nomenclatural species where they can most readily be found again, he has chosen the former course.

He has placed all species with one septate ascospores, whether hyaline or brown and with or without appendages, in *Melanconis* (with twenty-five accepted species). In its typical section, the A-conidia are one-celled, brown and superficial; in one species and three varieties, these conidia are hyaline; in two other species they are immersed. In other species again, the A-conidia are many-septate, brown and either superficial as in *M. modonia*, or immersed as in *M. thelebola*. In *M. aucta*, the ascospores themselves, after extrusion from the perithecium, become brown and three-septate, and its conidia are believed to be hyaline, many-septate and immersed.

*Pseudovalsa*, with three accepted species, is here characterized by its perithecia being immersed in a fuscous stroma, its ascospores several-septate and unappendaged, and its conidia several-septate and superficial. The ascospores are hyaline in one species and brown in the others.

*Prosthecium* with seven accepted species is defined as lacking a fuscous immersed stroma about its perithecia and as having several-septate appendaged ascospores, and several-septate immersed conidia. The ascospores are hyaline in three species and brown in four.

Other genera mentioned have received species excluded from *Melanconis* and *Pseudovalsa*; they are the invalid *Calospora* of Saccardo's use (its forty species are assigned to other genera); *Titania*, characterized by its one-spored asci, and only known from the type collection of its type species; *Phragmodiaporthe*; and *Massaria*, *Aglaospora*, *Thyridaria* (monographed elsewhere) and *Pseudotrichia*. The last four genera are segregated by the presence of numerous filiform, persistent 'paraphyses', a character which some workers accept as diagnostic of the family Pseudosphaeriaceae. Dr Wehmeyer holds that the case for maintaining such a family is not yet proved.

E. W. MASON



*Plant Science Formulae.* By R. C. McLEAN and W. R. IVIMEY COOK.  $8\frac{1}{2} \times 5\frac{1}{2}$  in. Pp. 203. London: Macmillan and Co. Ltd. Price 7s. 6d.

This useful book contains many of "the items of practical information which...are needed from day to day" in a botanical laboratory. Its justification is the collection of such items into a single handy volume, obviating the familiar troublesome search through book after book. The scope is wide: formulae for fixatives and preservatives; embedding, dehydrating, clearing and mounting media; chemical and micro-chemical reagents; culture and nutrient solutions and media; solutions for volumetric analysis. In so small a book there must necessarily have been many problems of selection and exclusion, and specialists may not always agree with the course adopted. It seems a pity that no notes were added on the suitability of the various fixatives for particular materials, especially as such notes are given in the valuable chapter on staining methods; and the omission of the simple and effective safranin and light green method from the list of recommended combination stains is rather surprising. The chapter on the preparation of museum specimens should be of great service to many laboratories. It is difficult, however, to see what principles underlie the choice of tests included in the next chapter on Chemical and Micro-Chemical Reagents. Thus there is a test for pyruvic acid but not for acetaldehyde; for K and Mg but not for Fe or Ca; for nitrate but not for phosphate or sulphate; for glutathione but not for ascorbic acid. The micro-osazone (not ozazone) test is given, but not the more generally successful macro-test; and the Selewanoﬀ, but not the pentose-furfural test. On page 86, where the separation of chlorophyll pigments is described, it should be pointed out that the lower layer does not contain true chlorophyll, especially if alcohol has been used as an extractant. The final chapters on Photographic Reagents (including some good dodges for lantern slides) and Workshop and General Receipts (imperishable putty, paint for blackboards, duplicating jelly, luting wax, cleaning coverslips, etc.) are excellent.

In conclusion, it seems worth while to quote from page 5 that "it cannot be too strongly emphasised that all botanical material, whether for anatomical investigation by a junior class or for cytological research work, should be cut up and properly fixed...immediately it is collected, and not be left for hours or days in a vasculum before finally being plunged whole into a bottle of alcohol." This book should help to abolish the "pickled cabbage" procedure.

A. R. CLAPHAM

## STUDIES IN THE EFFECTS OF PROLONGED ROTATION OF PLANTS ON A HORIZONTAL KLINOSTAT

### III. PHYSIOLOGICAL REACTIONS IN THE HYPOCOTYL OF *LUPINUS ALBUS*

By E. D. BRAIN, F.L.S.

(With 1 figure in the text)

Changes in the growth and development of plant tissues, resulting from continual rotation in a horizontal position, have been described in previous papers (Brain, 1935, 1939).

The following series of comparative investigations were performed on *Lupinus albus* seedlings and deal with certain physiological reactions of the hypocotyl when plants are grown on a klinostat:

- I. Geotropic response.
- II. pH value of the cell sap.
- III. Suction pressure value of the cell sap.
- IV. Extensibility of the cell walls.
- V. Growth-hormone distribution.

#### METHODS

Plants were grown in a greenhouse in pots of soil. Constant temperature could not be maintained, but careful records of maximum and minimum temperatures and percentage humidity were kept. Seeds were germinated and the pots placed on the klinostat when the plants had started growth, before the hypocotyls were 2.5 cm. in height. The klinostats rotated once an hour and all the plants were kept equally moist. Pots of control plants, grown upright, were surrounded by cylinders of white paper to prevent any phototropic curvature. In all cases experiments with plants grown on the klinostat and control plants were performed simultaneously under identical conditions.

#### I. GEOTROPIC RESPONSE

Experiments described in a previous paper (Brain, 1926) have shown that when *Lupinus polyphyllus* seedlings were grown on a klinostat the shape of the cross-section of the hypocotyl was altered and the presentation time for geotropic stimulation in the intercotyledonary plane was reduced from 80 to 60 min. Hawker (1932) correlated this change with the shape of the statocytes which were more nearly isodiametric when the seedlings were grown on the klinostat. The area of

the sensitive wall in the intercotyledonary plane is therefore increased and the statolith efficiency greater, resulting in the presentation time being less than it is normally for the intercotyledonary plane. In *Cucurbita pepo* no change in shape of the cells occurred on the klinostat and no difference in the presentation time was found (Brain, 1926). When *Lupinus albus* seedlings are grown on the klinostat there is very slight alteration in the shape of the statocytes in the hypocotyl (Brain, 1939).

Geotropic experiments have been performed with hypocotyls when they were grown on the klinostat and measured over 4.5 cm. in height. Stimulation was effected by stopping the klinostat for 20 min. Control plants were stimulated by laying the pots horizontal for 20 min. and replacing them upright for the reaction time. The latent time represents the time elapsing between the beginning of the stimulus and the first movement visible to the naked eye. The angles of curvature were measured with a transparent protractor. The results, which are recorded in Table 1, show that the reaction to gravity was not affected by growth on the klinostat, and similar results were obtained whether the plants were rotated for the reaction time or removed from the klinostat and placed upright.

Table 1. *Geotropic response in Lupinus albus hypocotyl*

No. of plants used	Percentage response	Average angle of curvature	Average latent time in min.	Average growth per day in cm.
Series 1. Plants grown on klinostat and rotated for reaction time				
20	70	7°	105	1.3
		Control		
20	85	7°	100	1.2
Series 2. Plants grown on klinostat and placed upright for reaction time				
20	60	7°	101	1.0
		Control		
20	60	6°	99	0.79

Length of stimulus, 20 min. Temperature  $20 \pm 1^\circ \text{C}$ . Humidity 70-90 %.

## II. pH VALUE OF THE CELL SAP

To determine the pH value of the cell sap in the cells of the hypocotyl of *Lupinus albus* the range indicator method was used, as described by Small (1929). Hand-sections were cut from the upper part of hypocotyls grown on the klinostat and upright. All seedlings were over 4.5 cm. in height. The sections were washed in neutral water, dried on filter paper and mounted on slides in the indicator solutions.

A detailed description of the method and the indicators used may be found in Small's paper (1929, pp. 46-63).

The sections were kept in the indicator solutions for at least 60 min., washed again in neutral water, and examined under the microscope and the colour recorded. Control sections in each case were used as a check for the natural colour. The indicators used enabled a distinction to be made between ranges of reaction or pH of 6.2-4.0.

The results found for five different series of experiments are recorded in Table 2 and do not show any difference in the pH value for the cortex, pith or phloem of hypocotyls whether they are grown on the klinostat or upright. In both cases results varied between 5.6 and 5.9 for the cortex and between 5.6 and 4.8 for the phloem.

Table 2. pH value of cell sap for hypocotyl of *Lupinus albus* (over 4.5 cm.)

Series	Upright		Klinostat	
	Cortex and pith	Phloem	Cortex and pith	Phloem
1	5.6	5.2-4.8	5.6	5.2-4.8
2	5.6-5.9	5.2-4.8	5.9	5.2-4.8
3	5.9	5.6	5.6-4.8	5.6
4	5.9	5.6	5.9	5.6
5	5.6	—	5.9	—

A few experiments with *Helianthus annuus* hypocotyls were made and the pH value was found to be 5.8-5.6 for the pith and cortex and 5.2-4.8 for the phloem, both for plants grown on the klinostat and upright. This is the value which Small (1929) has quoted for *Helianthus* seedlings. Metzner (1934) found a difference of 0.09 in the pH value for the expressed sap from the two halves of geotropically stimulated *Helianthus* seedlings. He found the lower half of hypocotyls had a pH value of 5.3 and the upper half 5.39 as compared with 5.34 for upright plants. This difference may be due to his using another method for the determination in which the expressed sap is tested instead of the tissues being examined intact as in the indicator method.

### III. SUCTION PRESSURE VALUE OF THE CELL SAP

Stiles (1924) has defined the full suction pressure of a cell as equal to the osmotic pressure of a non-penetrating substance in which the cell undergoes no change in volume. The method used for these experiments was the equilibrium method described by Stiles (1924). Hand-sections were cut from the upper part of hypocotyls, which were over 4.5 cm. in height, and washed in distilled water. These were dried on filter paper and mounted on slides in different concentrations of sugar solution. The sections were cut from several different plants, and at least three were mounted on each slide. The slides were examined under the microscope immediately to make sure that the cells were turgid. After 40 min. they were examined again and the concentrations of the sugar solutions were noted in which alteration in the size of the cells was evident. The concentration of the sugar solution in which no change of the cells occurred was regarded as equivalent to the suction pressure of the cell sap. Several other series of experiments were performed using paraffin for washing the sections instead of distilled water and then transferring them to the sugar solutions, the method described by Ernest (1934); but identical results were obtained for both methods. Ernest (1934) has criticized the use of sections rather than strips of tissue for suction-pressure experiments, but as these experiments were of a comparative value it is felt that her



criticisms do not invalidate these results. The use of strips has also been criticized by Diehl, Gorter, Van Iterson & Kleinhoonte (1939), who found it more satisfactory to use sections of tissue.

Table 3 records results obtained for some of the series of experiments performed and shows a value equivalent to  $0.21M$  sucrose or  $5.46$  atm. in the cells of the cortex of upright hypocotyls as compared with  $0.17M$  sucrose or  $4.42$  atm. for klinostat plants. A decrease in the suction pressure of approximately  $1$  atm. ( $0.04M$  sucrose) is therefore exhibited in the cortical cells when plants are grown on a klinostat. It is interesting to note that Diehl *et al.* (1939) found differences in the osmotic value of cells from convex and concave sides of *Helianthus* hypocotyls which were treated unilaterally with growth-hormone paste, the values for the convex side being equivalent to  $0.02$ – $0.05$  (mol. glucose solution) lower than for the concave side.

Table 3. *Lupinus albus* hypocotyl (over  $4.5$  cm.)

Suction pressure, cane sugar ( $M$ )

Series	Upright	Klinostat
1	0.21	0.17
2	0.21	0.17
3	0.19	0.17
4	0.21	0.17
5	0.21	0.17

In 1, 2 and 3 distilled water was used for preparatory washing of sections. In 4 and 5 paraffin was used.

#### IV. EXTENSIBILITY OF THE CELL WALLS

Measurements of the extensibility of the cell walls have been made by two methods.

(1) *Macroscopic method.* Pieces of the upper part of hypocotyls (over  $4.5$  cm.) were cut  $5$  mm. in length and halved lengthways. These were placed in distilled water for  $1$  hr., dried on filter paper and then plasmolysed in a solution of  $50\%$  cane sugar, and their lengths measured after intervals of  $45$  min. The shrinkage noted was worked out as a percentage, and thus an approximate value for the extensibility of the cell walls was obtained. When replaced in distilled water the original length of the strip of tissue was regained. The results obtained, examples of which are recorded in Table 4, show that the average longitudinal extensibility of the pieces of tissue was greater in plants grown on the klinostat than upright.

(2) *Microscopic method.* Longitudinal sections were cut from the upper part of hypocotyls and placed in distilled water for  $1$  hr. so that the cells should attain their greatest volume. They were then removed and dried on filter paper and mounted on a slide in  $50\%$  sucrose solution. The slide was examined under the microscope, and several cells were then drawn, with the camera lucida, on squared paper. The same cells were again drawn when just plasmolysed after about  $40$  min. in the sugar solution. The two areas of the group of cells were measured and the percentage decrease in size calculated, which expressed the extensibility of the cell walls from the plasmolysed to the saturated state. Table 4 shows that the

percentage decrease for cells on the klinostat is greater than for upright plants, which means that the cell walls are more extensible when the plants are grown on the klinostat. This also corresponds with results of Diehl *et al.* (1939) for *Helianthus* hypocotyls after treatment with growth-hormone paste. An increase in the length-wise extensibility of longitudinal epidermal cell walls was shown by them after 2 hr. treatment with growth-hormone paste.

Table 4. *Lupinus albus* hypocotyl (over 4.5 cm.). Extensibility of cell walls

Series 1. Macroscopic method		
	No. of pieces	Percentage decrease in length
Upright	11	9.85 $\pm$ 1.12
Klinostat	38	14.6 $\pm$ 0.74
Series 2. Microscopic method		
	No. of cells	Percentage decrease in area
Upright	9	6.21 $\pm$ 1.05
Klinostat	24	10.8 $\pm$ 1.109

#### V. GROWTH-HORMONE DISTRIBUTION

Dijkmann (1934) has studied the production and distribution of growth hormones in the hypocotyl of *Lupinus albus*. His analysis has shown that the amount of growth hormone extracted from different parts of the hypocotyl varies with the age of the plant. Hypocotyls of 3 cm. yield equal amounts from 7 mm. segments of the upper, middle and lower parts, but other seedlings of 8 and 5 cm. yield 5.1 % less from the middle than from the upper part. Comparative estimations of the growth substance in seedlings grown normally and on the klinostat have been made by a straight-growth method which has previously been described (Brain, 1941). The hypocotyl was cut into segments of 5 mm. which were placed with lanoline on to the stumps of prepared pea seedlings. The growth substance which diffused out of the hypocotyl segments caused increase in length of the pea stumps which was measured with a millimetre rule or scale.

#### Method

Peas were grown in a greenhouse in pots of soil, and when three or four internodes high the internodes were measured separately and their growth recorded. The plants were placed in a dark box for 24 hr. before the experiments. They were prepared by cutting off the actively growing internodes at the node below which growth has ceased. This height was measured and recorded as *cut height* (1). The plants were then left for 30 min., after which the cut surface was dried with filter paper and covered with a layer of lanoline on which the segment of *Lupinus* hypocotyl to be tested was placed. A vertical millimetre scale was placed in the pot behind each pea and the cut height marked on it. The plants were then replaced in the dark box for another 24 hr., after which the cut height was again measured. In this way the growth substance which has diffused from the lupin segments causes growth in the pea epicotyl, after it has ceased to grow normally, and gives a means of measuring the growth substance which can be obtained by diffusion from the test material. Experiments to standardize the measurements

with known concentrations of indole-3-acetic acid solution show that an increase of 1 mm. in the pea stump is equivalent to the growth caused by a 3% agar block of 40 cu.mm. volume containing 0.5% indole-3-acetic acid solution, at a temperature of 72–54° F. and a relative humidity of 100%. According to Went (1937) 0.5% indole-3-acetic acid solution in a 10 mm. agar block at 24° C. and 85% relative humidity would produce 5° curvature in the Avena test. So the results for the pea stump would approximate to 5° curvature in the Avena test. It was therefore suggested that one *pea unit* should be that amount of growth substance which would produce an increase of 1 mm. in the pea stump at a temperature of 72–54° F. and 100% relative humidity.

Table 5 records the averages of results for sixty-six experiments.

Table 5. *Growth-hormone distribution in Lupinus albus hypocotyl (over 4.5 cm.)*

	Klinostat		Upright	
	1st segment	2nd segment	1st segment	2nd segment
No. of experiments	20	22	12	12
Percentage positive	90	91	81	56
Average amount of growth hormone in <i>pea units</i>	1.4	1.3	1.1	0.83

A comparison between the seedlings grown on the klinostat and upright show differences in the amount of growth substance obtainable in both cases. In upright plants the uppermost segments of the hypocotyl, 5 mm. below the cotyledons, yield an average of 1.1 unit of growth substance and the next segment below 0.83 of a unit. In klinostat plants the upper segment yields an average of 1.4 units and the next segment below 1.3 units. Also positive results were found in 81% of the upper segments and 56% of the lower segments in upright plants, but in the klinostat plants 90% of the upper segments and 91% of the lower segments were positive (i.e. yielded at least 0.5 unit of growth substance). Thus it appears that plants grown on the klinostat have more growth substance more evenly distributed than in upright plants of the same age. The distribution is more like that described by Dijkmann in younger seedlings. Some examples are shown in Table 6 which gives the results for twelve seedlings, six upright and six grown on the klinostat.

Table 6. *Lupinus albus seedlings*  
Temperature 72–54° F. Relative humidity 100%

Upright			Klinostat		
Height in cm.	Growth hormone in <i>pea units</i>		Height in cm.	Growth hormone in <i>pea units</i>	
	1st segment	2nd segment		1st segment	2nd segment
4.7	0.5	0.5	4.5	1.0	1.0
5.0	1.0	—	4.5	2.0	1.5
5.5	1.0	—	5.0	2.0	2.0
6.0	1.0	—	5.0	2.0	2.0
6.0	1.0	1.0	5.0	2.0	—
7.0	1.0	—	7.5	2.0	1.0

Dijkmann has estimated the amounts of growth hormone which are contained in 7 mm. blocks of lupin hypocotyls from the upper and lower halves of hypocotyls after being horizontal for 4 hr., and he finds, of the total amount present, 32.5% is in the upper half and 67.5% in the lower half, but the total amount is not influenced by the stimulus. He therefore assumes that it is the distribution of growth hormone and not its production which is influenced by gravity. He also considers that the cells of the hypocotyl themselves manufacture the growth hormone from food substance or a precursor substance which is supplied by the cotyledons.

When plants are grown on the klinostat and receive a prolonged gravitational stimulus there appears to be a definite increase in the amount of growth hormone which can be measured by diffusion from the hypocotyl. This is evident both by more growth hormone being found in the uppermost 5 mm. of the hypocotyl of klinostat plants and in the fact that there is not the same decrease in growth-hormone content in the lower part of the hypocotyl in klinostat plants as is found normally in plants of the same age.

Another set of experiments was performed to demonstrate the effect of the klinostat on growth-hormone distribution in the hypocotyl. When one cotyledon and the plumule are cut off the lupin hypocotyl makes a curvature toward the side without the cotyledon, since on that side the auxin supply is curtailed and growth retarded, while the other side continues to grow. When plants grown on the klinostat were similarly treated it was found that much bigger curvatures resulted which indicates a greater difference in the growth of the two sides of the hypocotyl than in upright plants. Purdy's method for estimating the difference in growth on two sides of a curved *Avena* coleoptile, as described by Went (1937), has been adapted for measuring the difference in growth on the two sides of the curved lupin hypocotyls. This is calculated from the formula  $d = tl/r$ , where  $l$  = the length of the part of the hypocotyl which bends,  $r$  = the length of the radius of curvature,  $t$  = the thickness of the hypocotyl, and  $d$  = the difference in growth on the two sides of the hypocotyl which is bent (see Fig. 1). The average of values for  $d$  calculated for normal plants is 2.16 mm., and for klinostat plants 4.3 mm., which shows a marked difference due to the effect of the klinostat.

Table 7 gives an example of one series of experiments performed. Diehl *et al.* (1939) performed parallel experiments on *Helianthus annuus* seedlings. When decapitated hypocotyls had growth substance in lanoline paste applied to one side of the hypocotyl curvatures resulted, due to the increased growth on one side of

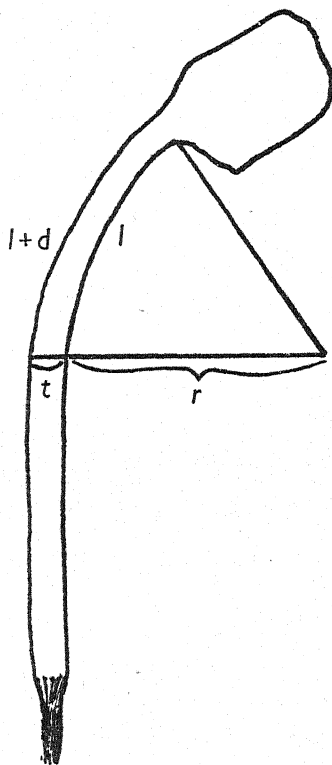


Fig. 1. Method for measuring the difference in growth on two sides of a curved lupin hypocotyl. For explanation see text.



the hypocotyl. If unilaterally treated plants were placed on the klinostat bigger angles resulted. This was explained as being due to the effect of gravity on the curving hypocotyl when some of the growth substance may spread to the concave side, whereas on a klinostat this condition is prevented and a bigger curvature results. I would suggest that the case of unilateral application of growth substance is comparable to the experiment of removing one cotyledon, because in both cases the growth-substance supply is altered on two sides of the stem, and Diehl's explanation could apply to this experiment as well.

Table 7. *Lupinus albus* hypocotyls. Cotyledon cut after 5 days on klinostat

Upright					Klinostat						
Height of plants in cm.	Angle of curvature after 3 days	<i>l</i> mm.	<i>r</i> mm.	<i>t</i> mm.	<i>d</i> mm.	Height of plants in cm.	Angle of curvature after 3 days	<i>l</i> mm.	<i>r</i> mm.	<i>t</i> mm.	<i>d</i> mm.
9.3	5°	48	72	5	3.4	9.5	50°	35	28	5	7.9
8.5	8°	55	155	5	1.7	8.4	25°	33	37	5	4.4
7.5	10°	42	120	5	1.7	8.2	30°	30	32	5	4.7
7.5	10°	38	82	5	2.3	7.8	25°	22	26	5	4.2
6.5	10°	35	80	5	2.2	7.5	20°	60	84	5	3.5

However, experiments described above have shown that the klinostat increases the elasticity of the cell walls, and Diehl has shown that application of growth-hormone paste also increases the elasticity of the cell walls. It would therefore also seem a possibility, that, on the klinostat, elasticity effects of the unilateral application would be increased, and so a greater difference in growth of the two sides of the hypocotyl result, giving bigger curvatures. Similarly, when the growth-hormone supply is impaired on one side the difference would be greater on the klinostat than upright. But from these experiments there does appear to be more growth hormone present in the hypocotyls of plants which have been grown on the klinostat and subjected to prolonged gravitational stimulus than in normal plants of the same age. An explanation of this may be that the klinostat reorganizes the distribution of the growth hormone in the stem, so that cells which normally cease growing have their activity prolonged and their growth-hormone production does not fall off so rapidly. This is an effect of prolonged gravitational stimulus and is not apparent in shorter periods when the redistribution of the hormone has been demonstrated by Dijkmann, but occurs as a secondary effect. Other instances of the same kind have been referred to in the previous papers of this series. On the klinostat the main roots of seedlings tend to grow longer and the side roots develop more slowly than in upright plants (Brain, 1935), and anatomical investigation showed that the sclerenchyma does not develop so rapidly in petioles of *Asplenium bulbiferum* on the klinostat (Brain, 1939).

#### DISCUSSION OF RESULTS

Reference has been made above to the results which other workers have obtained for pH value and suction-pressure value of the cell sap, extensibility of the cell walls and growth-hormone distribution in the upper and lower halves of the hypocotyls of seedlings which have been laid horizontal. In each case the results

for the lower side of the horizontal stem correspond with the results found for plants grown on the klinostat. This endorses the view that it is those changes which predominate in geotropic response and that they are brought about by a redistribution of growth hormone in the hypocotyl. In a horizontal plant which is prevented from bending by rotation on a klinostat greater growth rate in the shoot and decreased rate in the root, increased size of cells in the cortex and pith of the shoot, and decreased size in the cells of the radicle are evident. The period of active growth in certain cells seems prolonged on the klinostat. No difference in response to gravity has been found, neither in the size of the angle of curvature nor in the length of the latent period; neither did anatomical investigation show any changes in the distribution of statocytes when plants were grown on the klinostat.

It is of interest to note that the changes which have been described, i.e. greater extensibility of the cell walls and decrease in the suction-pressure value of the cell sap resulting in enlargement of the cells of the cortex and pith of *Lupinus albus* hypocotyls, closely correspond with the findings of Diehl *et al.* for the effect of applying growth-hormone paste to *Helianthus annuus* seedlings, which gives further proof that growth-hormone distribution is a fundamental process in geotropic response. These results may also be correlated with Borgström's theory of the transverse distribution of hormones (1939) which seeks to relate various physiological and morphological processes to the response of the plant to conditions which divert the normal course of auxin distribution into a transverse direction.

#### SUMMARY

1. Geotropic response, pH value of the cell sap, suction-pressure value of the cell sap, extensibility of the cell walls, and growth-hormone distribution have been examined in the hypocotyls of *Lupinus albus* seedlings which have been grown on a horizontal klinostat.

2. Geotropic response has not been found to be affected by growth on the klinostat. *L. albus* hypocotyls stimulated for 20 min. made an average curvature of 7° whether plants were grown upright or on the klinostat. The average latent time was 105 min. for klinostat plants and 100 min. for control plants.

3. The pH value of the cell sap was determined by the range indicator method, and the values found for both klinostat and upright seedlings varied between 5.6 and 5.9 for the cells of the cortex of the hypocotyl.

4. The suction-pressure value of the cell sap was found to be equivalent to 0.17M sucrose solution or 4.42 atm. for hypocotyls on the klinostat and 0.21M sucrose solution or 5.46 atm. in upright plants.

5. The extensibility of the cell walls was measured in two ways. (1) The shrinkage of pieces of hypocotyl in a plasmolysing solution of sucrose was measured, and the percentage decrease calculated for the klinostat was 14.6 and for upright plants 9.85. (2) The percentage decrease of the area of cells in longitudinal section was measured after plasmolysing in sucrose solution. This was found to be 10.8% for klinostat plants and 6.21% for upright plants.

6. Growth-hormone distribution was examined by two methods. (1) The amount of growth hormone which would diffuse out of 5 mm. segments of the

hypocotyl was measured by the increase induced in cut pea shoots when the lupin segment was applied on lanoline. Results showed more growth hormone diffused out of klinostat hypocotyls than upright plants. (2) When one cotyledon and the plumule are cut off the hypocotyl curves towards the side without the cotyledon. Plants on the klinostat made greater curvatures than upright ones, and the difference in growth on the two sides of the hypocotyl was 4.3 mm. on the klinostat and 2.16 mm. in upright plants.

7. Results for these experiments correspond with the effects of applying growth-hormone paste to *Helianthus annuus* seedlings, described by Diehl *et al.* (1939), and indicate the fact that differential growth-hormone distribution is a fundamental process in geotropic response and induces greater extensibility of the cell walls which received increased hormone supply.

My thanks are due to the University of Reading for the loan of a klinostat.

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## HARPOCHYTRIUM TENUISSIMUM KORSCH.

By FRANK W. JANE

*Department of Botany, University College, London*

(With 39 figures in the text)

The plant which forms the subject of this note was found in a tub in the grounds of the University College of North Wales, Bangor, in January 1941. It occurred chiefly on *Oedogonium*, but was also found upon *Microspora*.

The cells average  $2.7\mu$  in diameter, ranging from 2 to  $3.7\mu$ , but rarely exceeding  $3\mu$ ; normally their length does not exceed  $60\mu$ , but cells up to  $70\mu$  are not uncommon; those of a greater length, up to  $100\mu$ , were rarely seen. Thus, although in shape the plant is similar to Korschikoff's *Harpochytrium tenuissimum*, the cells are slightly thicker, and probably shorter. Korschikoff (1931) states that the cells of *H. tenuissimum* are up to  $120\mu$  long and at most  $2.5\mu$  thick; his material was, apparently, very limited in quantity, and his description of the plant is not very detailed; whether relatively short cells occurred, comparable in length to those of the Bangor plants, is not indicated in his paper; the description merely states that the largest cells were up to  $120\mu$  long. Of the other straight and erect species of the genus the Bangor plant bears some resemblance to *H. intermedium* Atkinson, from which it differs in being thinner and perhaps shorter, and in its apex, which is generally bluntly pointed like that of *H. tenuissimum*, and not tapering to a long point as in *H. intermedium*. Moreover, the young stages of the Bangor plant do not taper gradually from near the base to the apex, as in *H. intermedium*, nor are they of uniform diameter throughout their length; they possess a swollen basal region (Figs. 27-31). It is uncertain whether the young plants of *H. tenuissimum* also show a swollen basal region, but the plants figured by Korschikoff are both slightly swollen at the base, and it is not unreasonable to surmise that the young plants also exhibit this character, perhaps to a more marked degree.

Our present knowledge of the plants of this genus is far from complete, and it seems undesirable to establish a new species for the Bangor plants, although they certainly differ slightly from those which Korschikoff has described as *H. tenuissimum*; nevertheless, this divergence may well be due to environmental differences, and the Bangor plants are therefore referred to this species.

### STRUCTURE

The plant is more or less uniform in diameter throughout the greater part of its length (Figs. 1-6), although the basal region may be swollen (Fig. 2): the apex is generally somewhat pointed (Figs. 2-4), but is sometimes rounded (Figs. 1, 6), while at times the conical tip widens slightly at the extreme apex so that the plant terminates in a minute knob (Fig. 31). Rarely the plant is somewhat wider toward the apex than at the base (Fig. 6). In two young plants and an older one (Figs.



32-34) the apex was branched, but this is evidently a very rare condition, and does not appear to be comparable to the lateral outgrowing which sometimes occurs in the residual protoplasm of *H. Atkinsonianum* (Atkinson, 1903).

At its base the cell narrows suddenly to form a thin short stalk, which broadens out into a disk-like foot by means of which the plant is attached to an alga (Figs. 7, 7a); the length and thickness of the stalk are somewhat variable (Figs. 1, 3, 7). The attachment of the *Harpochytrium* to the alga is completely superficial; in fact, when attempts were made to stain and fix the material nearly all the *Harpochytrium* became detached from the algal filaments. There is, thus, reason for assuming that the plant is a saprophyte, and that it occurs on the 'host' alga as an epiphyte, and not as a parasite.

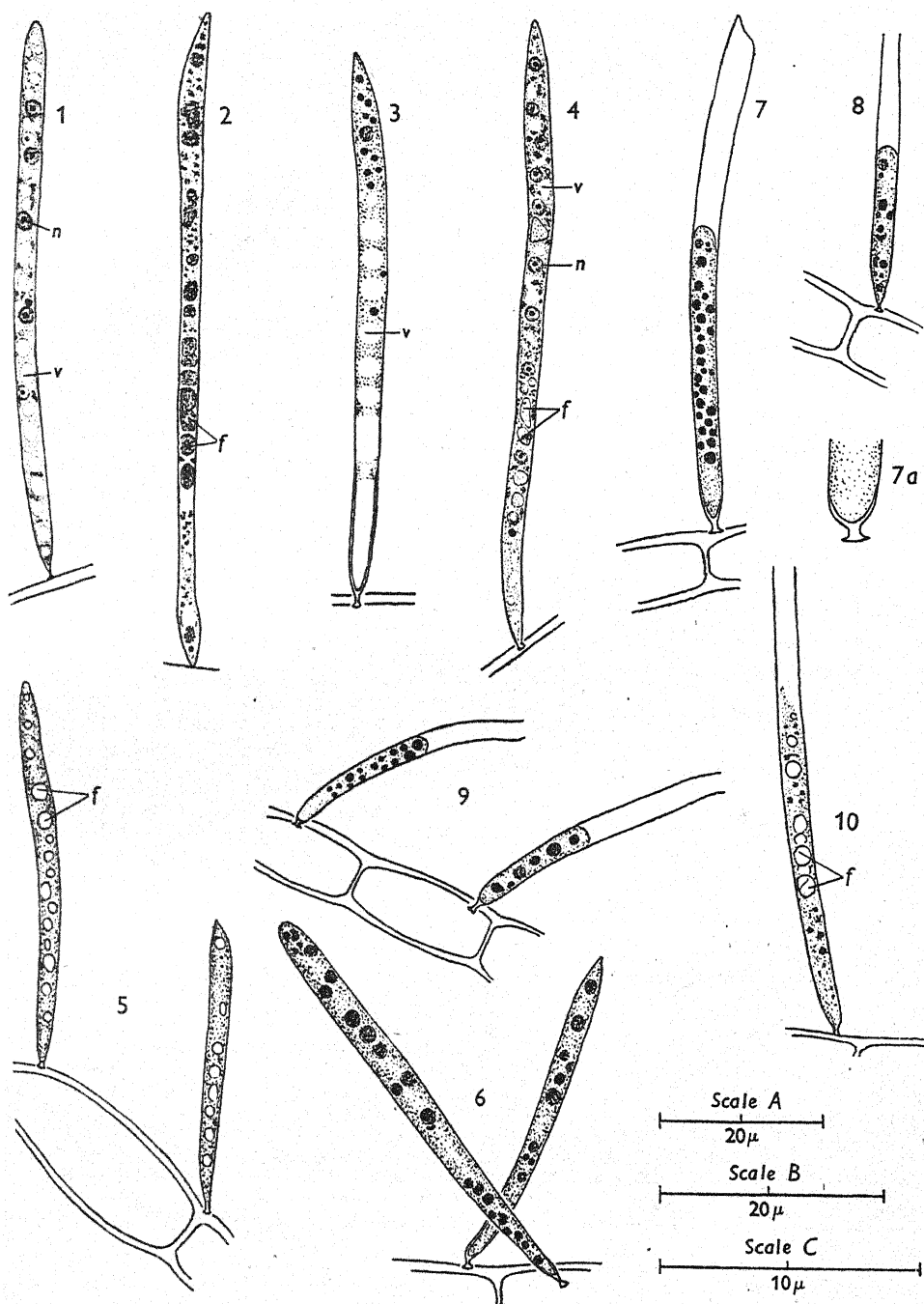
The cell wall is very thin, although it is thicker in the basal region than elsewhere (Figs. 7, 7a). It is readily stained by congo red: it gives a negative result when tested for cellulose, while the application of ruthenium red gave no indication of the presence of pectic materials. The wall did not show a lamellate structure, even when treated with aqueous and alcoholic potash solutions, nor was it possible to observe, either by direct examination or by treatment with strong chromic acid, that the wall was composed of two parts.

The cell varies considerably in different individuals. The cytoplasm is usually somewhat granular (Fig. 2), at times markedly so (Fig. 7): it may contain numerous small vacuoles (Fig. 1), at times a few larger ones (Fig. 3), which nearly equal the cell in diameter. Nuclei could not be made out in the living cell even with the aid of vital staining. They were demonstrated most satisfactorily by treating material preserved in about 3% formol with iron aceto-carmines for a lengthy period—at least overnight, or even for as long as a week. Even when so stained the nuclei are easily overlooked unless illumination is critical; they appear as little more than clear spherical vesicles, usually with a single, centrally placed nucleolus: as, moreover, their diameter is rather less than  $1.5\mu$ , it is not surprising that they are indistinguishable from the rest of the protoplasm in living material. A cell usually contains from one to four or five nuclei (Fig. 1), although as many as nine or ten may be observed in long cells (Fig. 5). Most of the nuclei occupy the upper part of the cell, where they are more or less evenly spaced: in uninucleate cells the nucleus usually lies in the middle of the cell.

Apart from granules, and the vacuoles, globules of fat are more or less abundant in the cells. These globules are dissolved out of the cell by chloroform, they readily take up such fat stains as sudan black and sudan blue, and are darkened by osmic

#### Legends for Figs. 1-10

Figs. 1-10. Figs. 1-6. Vegetative plants. Fig. 1. Plant of typical shape, with four nuclei; formol, iron aceto-carmines; scale B. Fig. 2. Plant with somewhat bulbous basal region; formol; scale A. Fig. 3. Plant with very vacuolate cytoplasm; methylene blue, vital; scale B. Fig. 4. Plant with at least nine, probably ten, nuclei; formol, iron aceto-carmines, then aqueous orange G; scale B. Fig. 5. Small plants with abundant fat; sudan blue; scale A. Fig. 6. Two plants, of which the right-hand one is of more or less typical shape, while that on the left is unusual in having a bluntly rounded apex; scale A. Figs. 7-10. Plants from which zoospores have been liberated; all scale A, except 7a, which is scale C. Fig. 7a. Shows the base of Fig. 7 enlarged: in Fig. 10 the top of the protoplasm is naked, and zoospores have probably just been liberated; Nile blue sulphate. *f.* fat globules; *n.* nucleus; *v.* vacuole.



Figs. 1-10

acid. Treated with Nile blue sulphate they normally become pink, from which it is concluded that they consist of a neutral fat, although in a few of the plants tested with this reagent the globules stained blue, indicating that they sometimes consist of a fatty acid. The fat globules are generally large (Fig. 2), although smaller ones may occur (Figs. 4, 5). In actively growing cells fat globules are not abundant (but cf. sporeling, p. 96), if present at all, but in less active cells, and living cells have been kept under observation for some 10 weeks without exhibiting any change, the fat globules are usually numerous and relatively large (Figs. 11-13).

Tests for proteins gave no evidence that these substances are stored by the cells, but it should be pointed out that the smaller granules of the cytoplasm are of such dimensions as to render unreliable any colour test which may be made upon them; their nature could not be determined; nevertheless, there is no evidence that the cells store protein food reserves as well as fat.

#### RESTING STAGES

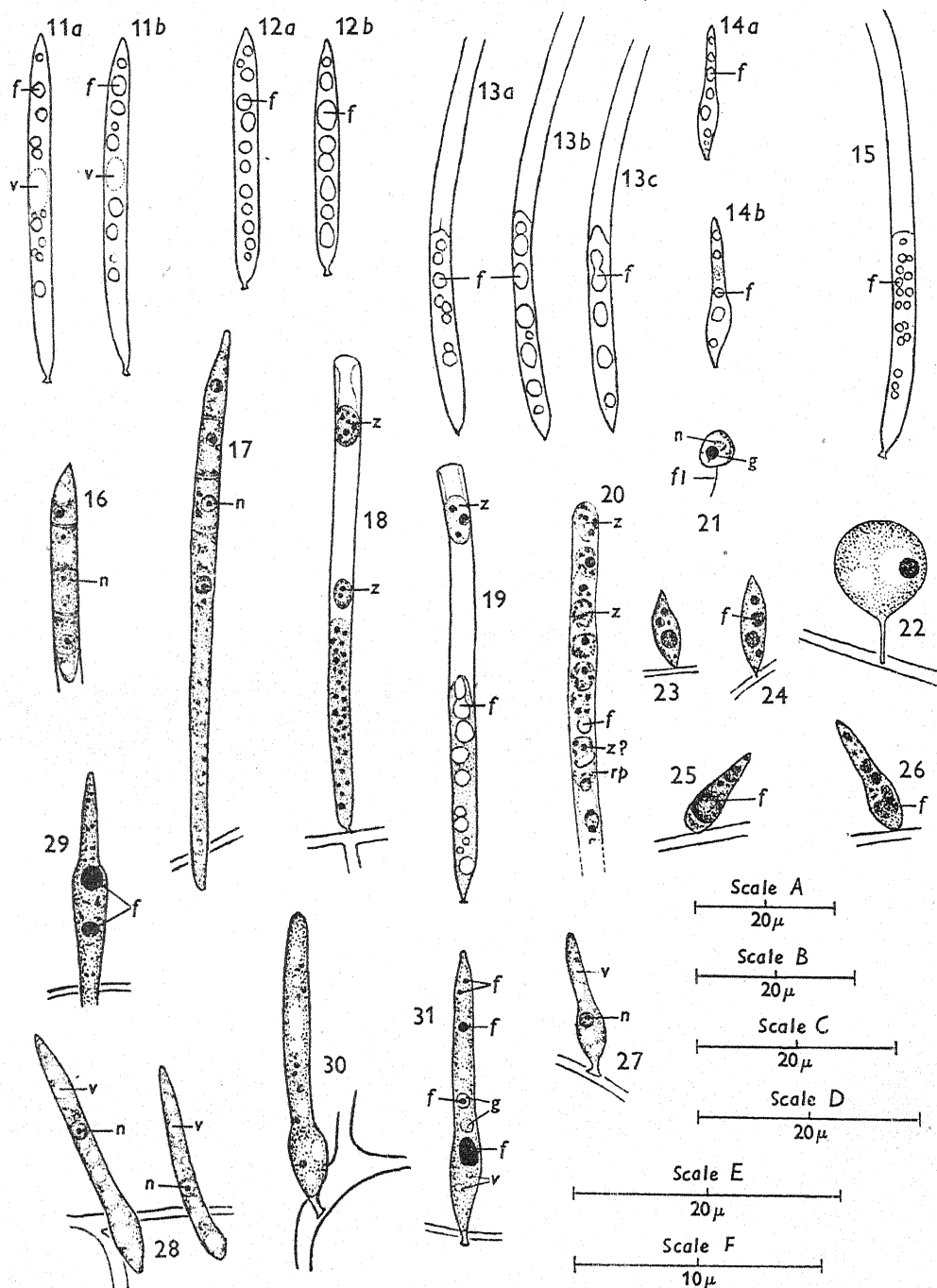
Attempts were made to study the growth and development of individual cells by using the lens-paper aquarium,<sup>1</sup> but without success, presumably because the conditions obtaining in such cultures were inimical to active growth. Nevertheless, in such cultures the fat in the cells usually assumed the form of large drops (Figs. 11-15), and in this condition the cells remained, without change—some were kept under observation for ten weeks. Evidently such cells were in a resting condition,

<sup>1</sup> This device, described by Walton (1915), does not appear to be generally known. It is essentially a mount in which the cover-slip is separated from the slide by a frame of lens paper of slightly smaller dimensions than the cover-slip. The material to be studied is placed in the centre of the frame, and the cover-slip mounted; the mount is allowed to dry out slightly, until water no longer extends beyond the edges of the frame, and the margins of the cover-slip are ringed with liquid paraffin.

The advantage of the lens-paper aquarium over the hanging-drop culture is that, if a *very thin* cover-slip be used, all parts of the mount can be examined with a 1/12 in. lens; for such work it is advisable to use methyl benzoate in place of cedar oil as the immersion fluid. The disadvantage of the lens-paper aquarium is that it is a closed aquarium, having no contact with the atmosphere; it is obviously necessary, therefore, to include in the mount both autophytic and holozoic organisms, and the aquarium will be successful only if there is a nice balance between the two; under such conditions organisms will sometimes live for months.

#### Legends for Figs. 11-31

Figs. 11-31. Figs. 11-15. Plants drawn from lens-paper aquarium cultures. Figs. 11a-14a and 15 drawn just after culture had been set up, Figs. 11b and 12b drawn 3 days later, Figs. 13b and 14b 5 days later; after 9 weeks Figs. 11b, 12b and 14b showed no appreciable change; in the plant shown in Fig. 13 the fat globules were larger and fewer (Fig. 13c), about a month after Fig. 13b was drawn, while about a month later the cell contained but two large fat globules; the plant shown in Fig. 15 showed no appreciable change 1 month later. All scale A. Figs. 16, 17. Segmentation of apical region to form zoospores; contents of lower part of plant shown in Fig. 16 were probably not viable; formol, iron aceto-carmin; scale D. Figs. 18, 19. Living plants containing zoospores in distal end; scale B. Fig. 20. Upper end of plant with at least seven zoospores; formol, iron aceto-carmin, Sudan blue; scale D. Fig. 21. Zoospore; 5 hr. earlier this was spherical and motile; Noland's solution; scale C. Fig. 22. Zoospore beginning to germinate; neutral red, vital; scale F. Figs. 23, 24. Very young sporelings; formol; scale C. Figs. 25, 26. Rather older sporelings; distal end now narrower than basal region; formol; scale C. Fig. 27. Young sporeling, uninucleate; formol, Delafield's Haematoxylin; scale D. Fig. 28. Two somewhat older, but still uninucleate sporelings; formol, iron aceto-carmin; scale D. Figs. 29-31, older sporelings, Figs. 29 and 31 stained in Sudan black; scale E. *f.* fat globules; *fl.* flagellum; *g.* granules of undetermined nature; *n.* nucleus; *rp.* residual protoplasm; *v.* vacuoles; *z.* zoospore.



Figs. 11-31



although occasionally in such cells (Fig. 15) smaller and more numerous fat globules were found. It is further evident that very young plants, as well as older ones, can assume this condition (Fig. 14).

Whether the plant adopts this method of passing through adverse conditions in nature could not be ascertained. Certainly the *Harpochytrium* disappeared from the source of supply in the early spring, and if any plants remained their number was small, for none was found after a thorough search.

#### REPRODUCTION

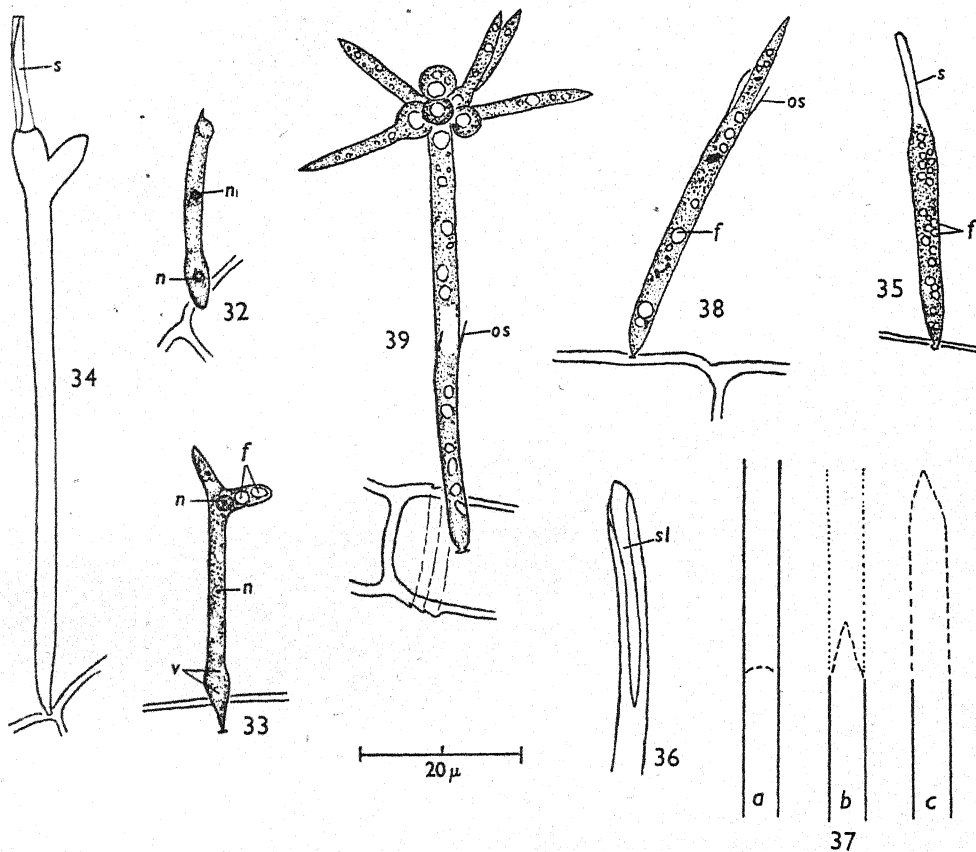
It is characteristic of the genus that only the upper part of the cell is used in the production of zoospores, the lower half continuing to grow and its upper half in its turn giving rise to zoospores. In *H. tenuissimum* the zoospores may be produced in the shorter, as well as in the longer cells (Figs. 7, 9); zoospore formation is initiated by the transverse segmentation of the protoplasm of the upper part of the cell; each segment, of which there are usually from four to six (Figs. 16, 17), is uninucleate, and gives rise to a single zoospore. To judge from the few cells which were found in this condition, the lower part of the cell has a single nucleus, and its protoplasm is not separated from the segmenting region by a transverse wall (Fig. 10). This transverse wall is formed later. The apical segments round off somewhat to form the ovoid zoospores (Figs. 18-20), which are liberated by breakdown of the apex of the cell. No movement was observed in zoospores in the sporangium. Although plants were kept under observation at every hour of the day and night, the author did not succeed in observing zoospores actually escape from the sporangium, and it was not, therefore, possible to follow the behaviour of the zoospore from the time of leaving the sporangium until it settled on an algal filament and produced a recognizable *Harpochytrium* plant. Nevertheless, one motile cell was observed which was undoubtedly a *Harpochytrium* zoospore; it remained motile for some 5 hr., finally attaching itself by the tip of its single flagellum (Fig. 21). Fig. 22 shows an older zoospore in which the flagellum has become shorter and thicker; it seems evident that this structure, by further shortening and thickening, gives rise to the short stalk and attaching disk; the zoospore now begins to elongate, while distally it grows out into a somewhat narrower cylindrical region; which, by elongation and increase in diameter, gives rise to the mature plant. Young plants are characterized by their obviously swollen basal region (Figs. 25-31) and even in an adult plant a slight basal swelling may remain (Fig. 2); this feature is well shown in Korschikoff's drawings. Very rarely the apical end of the cell increases to a diameter which exceeds that of the basal region (Fig. 6).

Sporelings are at first uninucleate; they often contain a considerable amount of fat, although this is not invariable; the cytoplasm may be markedly granular, and may contain large vacuoles (Figs. 25-31).

A unique plant is shown in Fig. 39, in which the zoospores have germinated at the mouth of the cell. It is thus apparent that at times the period of motility of the zoospores is very short, and that the length of time during which the zoospore remains active varies. In this respect the plant seems to be comparable with a species of *Saprolegnia* described by Lechmere (1910), who noted all stages

between the germination of non-motile spores in the sporangium, and the production of the normal type of *Saprolegnia* zoospore.

In the plant under present consideration the original wall is seen at *os*. Unfortunately, this part of the plant was by no means clear, and it is uncertain whether



Figs. 32-39. All figures are at same scale. Fig. 32. Young binucleate plant, with branching at very early stage; formol, iron aceto-carmin. Fig. 33. Young binucleate plant showing branching; formol, iron aceto-carmin. Fig. 34. Older branching plant which has already liberated zoospores; the old sporangium wall (*s*.) is beginning to decay; formol. Fig. 35. Plant which has liberated zoospores; the sporangium wall is beginning to collapse; living. Fig. 36. Apex of plant after liberation of zoospores, showing splitting of sporangium wall preceding disorganization. Fig. 37. Diagram to explain probable method of growth of cell wall (for explanation see text). Fig. 38. An unusual plant in which active growth of the residual protoplasm has occurred before the old sporangium wall has broken down. Fig. 39. Plant which, after proliferation has liberated a second batch of zoospores, which have germinated at the mouth of the cell. *f*. fat globules; *n*. nucleus; *os*. remains of old sporangium; *s*. wall of sporangium; *sl*. slit in wall of sporangium; *v*. vacuoles.

the part above this region arose as a result of branching (as in Fig. 34), or was a simple proliferation. In either case the second batch of zoospores to be set free germinated at the mouth of the cell; this new cell, however, is seen to be full of protoplasm, indicating a proliferation, preliminary to the liberation of the third batch of zoospores.

Plants from which the zoospores had been liberated were very common (Figs. 7-10); it was somewhat puzzling, therefore, to find little activity of the residual protoplasm; evidence of proliferation was extremely rare, and was, in fact, confined to the plant shown in Fig. 38, and to the unusual plant just referred to (Fig. 39). The explanation of this rarity of evidence of proliferation may be that after the zoospores have been liberated the residual protoplasm of the cell passes into a resting condition, and that some time elapses before zoospore formation again takes place. It is true that the protoplasm of such plants sometimes contains abundant fat globules, and this, it has been noted, is an indication that the plants are not growing actively. It is known that such plants will remain in the resting condition for some weeks: but the residual protoplasm is not always rich in fat, and it is probable that it often starts to grow actively again soon after the liberation of zoospores. The author is of the opinion that the very thin cell wall of the sporangial region collapses and becomes disorganized very soon after the zoospores have escaped. Early stages of this postulated collapse are shown in Figs. 34-36. The naked part of the residual protoplasm becomes covered by a wall (Fig. 37*a*, broken line), which of course is in connexion with the original wall of the plant; this elongates with the lengthening residual protoplasm, and, with the collapse of the old sporangium wall, forms the upper part of the wall of the cell (Fig. 37*a, b*, where the new wall is shown by a broken line, the old sporangial wall by the dotted line). In so thin a wall it would be extremely difficult, if not impossible, to detect the point of junction between the old wall and the new. At times (Figs. 38, 39) the old wall proves more resistant and remains after the residual protoplasm has elongated considerably, or even, in part, after the second batch of zoospores has been liberated.

#### THE TAXONOMY OF THE GENUS *HARPOCHYTRIUM*

While *Harpochytrium* was previously regarded as a chytridiaceous fungus, doubt was cast on the validity of this assumption by Wille at the beginning of the present century; he suggested that the plants were, in fact, saprophytic algae. Scherffel (1926) described, under the name of *H. viride*, a tubular algal form with a single, large chromatophore; this plant exhibits the proliferation of the sporangium regarded as so characteristic of *Harpochytrium*, and is, unquestionably, a xanthophycean alga. Pascher's *H. Scherffeli* has a somewhat similar habit, and is, no doubt, closely related to *H. viride*; its cells possess several chromatophores. The discovery of *H. viride* seems to have afforded sufficient justification for the assumption that the remaining, non-pigmented species of *Harpochytrium* are saprophytic, xanthophycean algae, although Pascher (1938) cautiously ventures the opinion that there may be, within this alleged genus, species perhaps not genetically related; he suggests that it may be advisable to separate the green and the colourless species, and in his key separates the obvious algal forms as a subgenus, *Chytridiochloris*, retaining the name *Harpochytrium* as a subgenus to embrace the colourless species.

Our knowledge of these plants is very incomplete, and more detailed studies are required before their relationships can profitably be discussed. Unfortunately, they appear to be of rare occurrence, and there would seem to be little prospect of one investigator obtaining a sufficient variety of material to make the needful

monographic investigation. It is, therefore, greatly to be desired that the plants should be investigated as completely as possible when they are encountered; the results of the present investigation are presented in their somewhat incomplete form in the hope that they may stimulate such investigations as the opportunity arises.

It is difficult at present to suggest any criteria which will enable us to distinguish with certainty between a chytridiaceous fungus and a colourless xanthophycean alga. Fritsch (1935) states that the cell wall of the Xanthophyceae is *often* rich in pectic compounds and that it is *frequently* composed of two equal or unequal pieces overlapping at their edges; but it is apparent that at present, at least, these features cannot be regarded as *invariably* associated with the Xanthophyceae. If, however, the obviously algal members of a genus, for example *Harpochytrium*, show these characters, it might reasonably be expected that the same state of affairs would exist in related, non-pigmented species. In *Harpochytrium* there is no information at present as to whether the cell wall of the pigmented species has these features, but in *H. tenuissimum* no evidence could be obtained that the cell wall contained pectic compounds, or that it consisted of two overlapping parts. If, when an opportunity presents itself to study the cell wall of the pigmented species, these characters are found to be absent, there would appear to be some justification for associating the colourless and the pigmented species: at present, however, the evidence for the relationship is not convincing.

Of other features, the presence of fat in the cells of *H. tenuissimum* is no more an algal than a fungal character, nor is the single flagellum of its zoospore, while the proliferating sporangium, admittedly a peculiar feature, which might be held to indicate relationships in plants of so similar a habit as that exhibited by the green and colourless species of *Harpochytrium*, is not unique; it is seen, for example, not only in the xanthophycean *Dioxys* but also in the chytridiaceous *Cladochytrium*, and again in members of the Saprolegniaceae. Possibly the germination of the zoospores at the mouth of the sporangium, of which a solitary instance is cited in *H. tenuissimum*, might be adduced as evidence of xanthophycean affinities, since it occurs in certain species of *Ophiocytium* (*Sciadium*); but analogous, if not strictly comparable, examples are seen in *Chlorangium* among the Chlorophyceae, while in *Achlya* the first-formed zoospores encyst at the mouth of the sporangium.

It is, perhaps, pertinent to ask, therefore, whether homoplasy is not a sufficient explanation of the similarity between the pigmented and colourless species of *Harpochytrium*: at least, sufficiently striking examples of this phenomenon may be cited (e.g. *Characium* and *Characiopsis*, *Protosiphon* and *Botrydium*). In the present state of our knowledge of the genus, caution suggests the desirability of separating the two groups of species, raising to generic rank Pascher's subgenus *Chytridiochloris* for the reception of the pigmented species, which are undoubtedly xanthophycean algae, and retaining the name *Harpochytrium* for the non-pigmented species. The two groups may be related, but it must be admitted that the present study does not appear to lend support to this view.



## SUMMARY

1. The structure and reproduction of *Harpochytrium tenuissimum* Korsch., are described.
2. The systematic position of this plant is discussed; available evidence no more suggests that its affinities are with the Xanthophyceae than with the Chytridiales.
3. It is suggested that with our present imperfect knowledge of the genus, there is not sufficient reason for associating together the pigmented and the non-pigmented species of the genus, and it is proposed that Pascher's subgeneric name of *Chytridiochloris* be raised to generic rank for the reception of the green species, while the name *Harpochytrium* be applied only to the colourless species.

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## ON THE CAUSES OF REGENERATION AFTER LONGITUDINAL SPLITS

By R. SNOW

*Fellow of Magdalen College, Oxford*

(With 4 figures in the text)

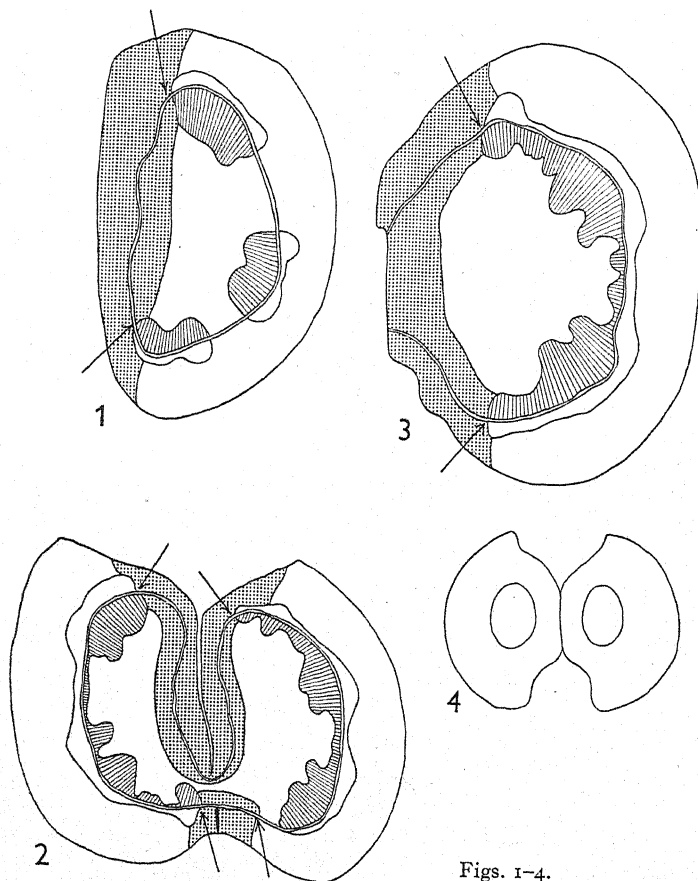
In many species if one makes a median longitudinal split in a young internode, without injuring the shoot apex, so that the cambium, as seen in transverse section, is divided into two half-rings, then, as was shown by Kny, cited by Vöchting (1892, p. 149), each of the half-rings becomes after a time rounded off into a complete ring through the formation of a new cambium on the inner side. Again, if a root tip is split longitudinally, the halves round themselves off by lateral regeneration, and in animals if a limb bud of an amphibian is divided longitudinally, the halves may form complete limbs. In all these examples, to which others might be added, it is surely of interest that regeneration is caused although nothing is removed: for this raises the question, What then exactly is the factor which sets the regeneration going? Accordingly, the writer has made a few experiments, which will here be reported and discussed, on the causes of this lateral regeneration in stems and root tips.

Vöchting (1892, p. 148) suggested an explanation of the rounding off of half-rings of cambia. From his experiments on transplantation in swollen roots of beet he had concluded that a cambium is caused to form internally to every surface, such as the external surface or a surface made by a cut, in a position and orientation which follow certain rules. A similar conclusion, though without the rules of orientation, had been reached previously by Bertrand (1884), who stated, without giving details, that in various species new cambia are formed around various obstacles and holes both artificial and natural, such as inserted needles, sclerites, secretory canals, cells containing crystals, and even intercellular spaces. Perhaps some of Bertrand's cambia were really phellogens. Vöchting went on to remark very cautiously that so far as he could see the rules which he had formulated covered all known examples of formation of cambia, including those in Kny's experiments.




However, it is certain that the natural obstacles and holes mentioned by Bertrand do not always cause cambia to form around them, and further, the experiments to be reported here will show that in young stems it is not every kind of artificial obstacle or hole which causes a cambium to form, so that the question will arise whether the cambial regeneration in split stems may really be due to some other cause.

The present experiments were made mainly on young hypocotyls of sunflowers. For the repetition of Kny's experiment these were split in a median longitudinal plane for a distance of about 1.0 or 1.5 cm. near their upper ends, usually when they had reached about half their final length or a little more, and the cut surfaces were

washed and vaselined, and a piece of mica was placed between them. After 3 or 4 weeks the split regions were examined by transverse sections, and it was regularly found that in each half a new cambium had been formed internally to the cut surface so as to connect the cut edges of the old cambium, in the manner shown in Fig. 1. The edges of the old cambium must have formed new patches of tissue by proliferation, since they no longer reached to the cut surface. Also the cells of the pith had grown and divided in the zone between the new cambium and the cut surface, and



Figs. 1-4.

Figs. 1 and 3 single halves and Fig. 2 both halves of split sunflower hypocotyls drawn in transverse section under projector, but semi-diagrammatic. Fig. 4 a split root-tip of *Vicia Faba* drawn free-hand.  
 Xylem.  Region of new cell divisions.  Cambium. ↓ ↓ Junctions of old and new cambia. Phloem left unshaded.

for a little distance internally to the new cambium, but the new cells so formed were much wider than those of the cambium. This new growth in the pith varied very much both in quantity and quality.

Young epicotyls also of *Phaseolus multiflorus* were split in the same way, and in these the cambial regeneration was found to be slower and more erratic. From examination of these epicotyls and also of those of the sunflower hypocotyls in which the regeneration was slowest, it appeared that the pith cells began to grow

and divide at an earlier stage, and that the new cambium was formed a little later within the zone of new divisions. It also appeared that new cambia were formed soonest close to the edges of the old cambium, and that from these positions they spread across internally to the cut surface until they met somewhere about the middle, though this is hardly what would be expected if the formation of the new cambium were somehow caused by the cut surface.

In some of the sunflower hypocotyls the halves were not separated with mica or vaseline, but were pressed together with strips of plasticine placed round the outside. When they were examined later by transverse sections, it was usually found that the cut edges of the old cambial ring had reunited completely, and there was then never any sign of a new cambium in the pith, and usually there were no divisions of the pith cells at all. Occasionally there were a few irregular divisions in the pith on each side of the line of the split, which was still made visible by the brown wound scar, but even these divisions were much fewer than when the halves were kept separated. In one hypocotyl it was found that at one level the halves of the old cambial ring had reunited, while in the pith the halves had gaped apart: yet even here there was no new cambium round the hole in the pith, but only a few irregular divisions of the pith cells. In another hypocotyl the halves of the cambial ring had reunited on one side only, and except on this side the halves of the pith were still separate. In this one (Fig. 2) a new cambium extended into the pith from the edges of the old cambial ring only on the side on which these had failed to reunite, so that it connected these edges by a loop, in some places rather feebly developed, reaching across nearly to the far side of the pith. Controls with mica strips between the halves showed that the pressure of the plasticine made no difference to the regeneration.

These observations strongly suggest that the factor which causes a new cambium to form in the pith of a young stem after a split is not the wound surface in the pith, but the permanent interruption of the old cambial ring. But to test the point further the following experiment was performed. A sunflower hypocotyl was split in the usual way and a strip of mica was inserted which was only so wide as to cover most of the pith without reaching to the xylem on either side. In order to see that the mica strip was correctly placed covering the pith only, it was necessary to cut through one of the split halves at the base and to lift up the flap so formed while inserting the strip. The halves were then pressed together again with plasticine. The mica strip extended lengthwise over nearly the whole length of the split. After 5 weeks, a longer period than usual, the split region was fixed in alcohol, the mica strip was drawn out from one end, and on examination it was found that the cut edges of the old cambium had reunited and that in the pith there was a slit-shaped hole where the mica had been. But around this hole there was no new cambium, and only a very narrow zone of irregular divisions of the pith cells, about three cells deep. In four other hypocotyls which were split as controls the halves were kept completely separated, and after about the same period all the eight halves had regenerated new cambia.

These results therefore confirmed the indications that the cambial regeneration in the pith of young split stems is not caused by the new surfaces in the pith, but by the interruption of the old cambial ring. The regeneration appears further to



follow the rule that when the cambial ring is permanently interrupted by a longitudinal cut or obstacle of any kind, new cambia start from the cut edges of the ring, turn laterally inwards through the pith, and continue to extend until they meet. Since the new cambia are apparently obliged as a rule to keep to some minimum distance from the wound surface (see Fig. 2), the surface does in that way take part in determining their position, though it is not the cause of their formation. A further indication that the new cambia extend gradually from the cut edges of the old one is that in a few of the halves of the permanently split hypocotyls, at some levels of the split, the new cambia had from some unknown cause turned outwards at two points so as to abut directly on the cut surface. An example is shown in Fig. 3. Where this had happened there was no new cambium in the region between these two points, so that it seemed that the new cambia had been prevented from extending further by running up against the cut surface.

An incidental question of some interest is what happens just above and below the split. In transverse sections of split hypocotyls made at these levels after about 5 weeks it was seen that the new cambia everywhere ended suddenly at just about the levels of the tops and bottoms of the splits, so that they must have extended roughly horizontally from the cut edges of the old cambia, and hardly at all in obliquely upward or downward directions. But in longitudinal sections it was seen that the new cambia were continued upwards and downwards by short stretches of irregular cell divisions which just arched over the tops and bottoms of the splits and gave the impression of being forerunners of new cambial zones. So it is quite probable that after still longer periods the new cambia would just have arched over the splits above and below.

Since it has now been concluded that the causal factor leading to the lateral regeneration of the cambia in split internodes is the interruption of the old cambial ring, it next becomes necessary to consider what may be physiologically the manner in which this interruption works. If the interruption of the cambial ring by a longitudinal split leads to certain effects, it seems that previously some process must have been taking place in the ring in lateral directions, and that it is the interruption of this process which causes the effects. For if there were no such process, the mere fact of morphological interruption would surely make no difference. It may of course be said that a morphogenetic field of some kind is interrupted by the split. But the conception of a morphogenetic field, even if convenient, is itself at present only descriptive and needs to be interpreted physiologically.

To make the discussion less abstract, it may be suggested that the normal process which is interrupted by a split in a cambial ring may be a movement of some process or substance in both directions round the ring. This would bring the lateral cambial regeneration into line with the increased cambial growth and formation of roots above horizontal cuts in stems, which are due to the interruption of the normal downward flow of auxin.

The next step therefore is to look for evidence of such a laterally moving process in the cambial ring, and one way of doing this is to try to determine whether the process depends on the movement of some active substance capable of diffusing out of the cells. An experiment for this purpose is readily suggested by the fact that when the halves of split cambial rings reunite, no new cambium is formed in the

pith. Will then a new cambium be formed if the halves are pressed together in moist contact, but somehow prevented from reuniting?

In order to test this point, six sunflower hypocotyls were split in the usual way, the cut surfaces were washed, a strip of moist linen was placed between the halves, and the halves were pressed together with plasticine. From time to time the plasticine was refashioned to keep it from being cracked by the growing parts. After 4 or 5 weeks it was found that strong new cambia had been formed in the pith in the usual way, no less strong than in controls with mica inserted in the split, so that the moist contact of the halves had made no difference.

These results are by themselves hardly adequate to exclude completely the possibility that the split acts by interrupting the lateral movement of a hormone, but they are supported by some rather similar experiments on split root tips to be reported below. Naturally it remains possible that the split may act by interrupting a laterally moving process of some other kind.

Incidentally, it may be remarked that a tendency to form new cambia as a result of some influence spreading from the edges of existing cambia would explain also the normal formation of interfascicular cambia.

Just the same questions arise with regard to the regeneration of longitudinally split root tips. For the halves round themselves off into complete new tips by growth on their inner sides, so that here also regeneration is set going though nothing is removed. Thus in split roots of *Vicia Faba*, for example, the halves of the apical meristem round off completely in a few days, and a little higher up also, at distances from about 0.3 to 1.0 mm. above the apical meristem, the halves round off, but here not completely. For at these slightly higher levels it is mainly the halves of the pterome or stele and of the inner cortex which round off, while the outer cortex scarcely does so (see Fig. 4). Through this regeneration the halves of the stele become not only rounded off, but also separated by parenchyma continuous with the old cortex, which is formed between them, as the figure shows. The halves regenerate at these slightly higher levels even if the split is not continued down through the apical meristem, as Němec mentions (1905, p. 250). The whole process is described in detail by Němec (1905, p. 134 seq.).

The same considerations, therefore, as have already been set out in regard to the lateral regeneration of cambia made it desirable to try to determine first whether the split root tips could be prevented from regenerating by being made to reunite, and secondly, if so, whether this could also be achieved by means of moist contact without reunion.

The task of preventing regeneration by making the halves reunite was found to be much more difficult than it was with the split internodes. Young roots of *Vicia Faba* were used, and the only method which ever succeeded for certain was to keep the halves pressed tightly together by making the split tips grow down through the narrowest obtainable rubber tubing, after strengthening and further narrowing the tubing by winding fine wire round it. Even this method succeeded for certain only with two of the split tips. In one of these it was found after a few days that the halves had elongated strongly and were completely unified into a single root tip and root throughout, although the brown wound scar was still visible between the halves in some places. The single stele was well formed. In the other, which had also elongated

well, the very lowest part of the apical meristem was double for a distance of about 0.1 mm., but above this came a zone of about 0.4 mm. in which the halves were completely unified, having a single plerome or stele, while higher up again the degree of unification varied at different levels.

But in eight others of these split root tips the extreme tips of the halves were found to be separate, and higher up the halves had formed two pleromes or steles, which were rounded off and separated by considerable parenchyma on their inner sides. Yet in these also the pressure of the tubing had made the halves adhere firmly together, and the brown wound scar was broken in many places. Some of these latter tips were split under water and kept under water for some hours before being inserted into the rubber tubing, and this must have washed away most of the wound substances formed. Purest distilled water ('conductivity' water) was used, since it had been found that under this water very little film or scar, if any, is formed on the wound surfaces; and with less wound scar diffusion from one half to the other will be easier. The evidence that regeneration does not depend on a wound substance is confirmed by another experiment in which a root tip was split under conductivity water, and one of the halves was removed to allow the water to wash the remaining half more freely. The remaining half was kept all the time under conductivity water, which was changed daily, but it regenerated just as well as usual. Its cut surface showed scarcely any film or scar.

In other split root tips in which the halves were not strongly pressed together, they regularly regenerated on their inner sides, although often they remained in good moist contact and adhering and were found to have only very little wound scar between them; and this scar was usually broken in many places. Many of these tips were split under conductivity water and were kept under it for some hours. The tips were then often dipped, with the halves adhering, into melted cocoa butter, which helped to hold the halves lightly together. They were then transferred to damp air, and water was supplied through the cotyledons. Again in many of the tips the root caps, and in some of them the apical meristems also, were left unsplit, to hold the halves together. But none of these arrangements ever prevented the halves from regenerating. Since regeneration was so persistent even when reunion was encouraged, there was no need to try experiments with moist contact but with reunion prevented.

It seems unlikely that all the regenerations of the half root tips, in spite of good moist contact and adhesions between them, can have been due merely to some defect in the conditions of contact. The results are surely enough to make it probable that the splits do *not* cause regeneration by interrupting the normal lateral flow of any hormone capable of diffusing out of the cells: and if this is so, the same is probably true for the split stems also. Yet for the reasons already given with regard to the split stems it seems necessary to suppose that the splits do act by interrupting a laterally moving process of some kind; and this process must therefore apparently be one for which protoplasmic continuity is needed. As to the two root tips in which the halves actually were prevented from regenerating by being pressed tightly together, it may be supposed that in these the halves quickly grew together again and re-established the protoplasmic continuity.

The inferred process of which the interruption by a split leads to regeneration

may now be regarded in a more simple and positive way as a process which normally maintains the morphological unity of the organ in the lateral directions. If the nature of such a process could be discovered, it would become possible to interpret physiologically a morphogenetic field. The conclusion that the lateral regeneration of split root tips is due to the interruption of some normal laterally moving process agrees well with the conclusion reached by Němec (1905, p. 362) that the regeneration of a root tip after decapitation is mainly due to the interruption of some correlative influence normally exerted by the tip upon the zone just above it. The conditions leading to lateral regeneration are also discussed by Němec (1905, p. 248 seq.).

One more set of experiments was performed to find out roughly what is the minimum distance for which a root tip must be split in the zone just above the apical meristem in order that the halves may regenerate. For this purpose root tips were split or pricked through transversely in the region from 0.3 to 0.5 mm. above the tip of the apical meristem, first with a fine-pointed scalpel, and then, when this was found to cause regeneration, with a steel needle and with progressively finer glass needles. Rather surprisingly it was found that a transverse prick through this zone with a glass needle only  $60\mu$  thick was regularly enough to cause regeneration. For the halves of the plerome rounded off into two pleromes or steles at the level of the prick, and considerable parenchyma was formed between them. This result tends to support the conclusion that lateral regeneration is not due to the interruption of a laterally moving hormone. Pricks with still thinner glass needles, only  $25\mu$  thick or less, did not cause regeneration.

#### SUMMARY

1. In young stems split longitudinally the halves of the cambial ring are known to become rounded off into two new rings by regeneration on the inner sides. Also in split root tips the halves round themselves off by regeneration on the inner sides. Since the splits do not remove anything, the question is raised, What is the causal factor which starts the regeneration?

2. Evidence is offered that the causal factor is neither a wound substance nor, as has been suggested, the formation of a new surface. It is shown that in split stems it is the interruption of the continuity of the old cambial ring in the transverse directions.

3. It is argued that if the splits cause regeneration by interrupting transverse continuity, then they must interrupt some process which normally takes place in the transverse directions and maintains the unity of the organ.

4. Evidence is offered indicating strongly that this transverse process is not the movement of a hormone capable of diffusing out of the cells, and that it needs continuity of protoplasm.

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## FURTHER EXPERIMENTS ON WHORLED PHYLLOTAXIS

By R. SNOW

*Fellow of Magdalen College, Oxford*

(With 13 figures in the text)

### I. INTRODUCTION

In a previous paper (Snow & Snow, 1935) we reported experiments designed to test whether the difference between whorled and spiral phyllotaxis is due to some intrinsic regulating property of the stem apices of species with whorled phyllotaxis, or whether whorled phyllotaxis also can be explained on the basis of the rule which, according to our earlier experiments (1931, 1935), holds good for the spiral phyllotaxis of *Lupinus albus*. This rule is that each new leaf arises in the first space that attains both a certain minimum width and a certain minimum distance below the growing point, and it continues and extends the ideas of Hofmeister (1868), Van Iterson (1907) and others. An explanation by this rule belongs to the group of what Schoute (1938) has called 'junctional' theories, because the phyllotaxis is supposed to be built up by joining each new leaf on to the pattern of the older leaves or other lateral members just below it. As to the origin of whorled phyllotaxis, we set out the problem more fully previously (1935, p. 64).

In order to study the difference between whorled and spiral phyllotaxis, we previously (1935) split in a diagonal vertical plane stem apices of *Epilobium hirsutum* which have decussate phyllotaxis (that is, alternating whorls of two), with the result that the halves usually developed spiral phyllotaxis after regenerating. We concluded (1935, p. 91) that the way in which this happened strongly supported the opinion of Weisse (1894), Van Iterson (1907) and others that the difference between spiral and decussate phyllotaxis depends mainly or entirely on 'junctional' factors in the above sense.

We did indeed notice (1935, p. 92) that the apices of *E. hirsutum* had less tendency to settle down after an operation to a spiral phyllotaxis with constant divergence angles than had those of *Lupinus albus*, and also (1935, p. 79 seq.) that they occasionally returned nearly to decussate phyllotaxis. But we considered (1935, p. 92) that it would be premature to conclude that these differences were due to any regulating mechanism, and that if the facts were more fully known the differences might perhaps be explained on a purely junctional theory, in terms of some such factors as the different shapes and sizes of the young leaves and apices in the two species.

Subsequently, however, a study of the effects of another operation on *Epilobium hirsutum* raised again the question whether whorled phyllotaxis is to some degree regulated by some special process which acts not instead of the junctional factors, but in addition to them. Schoute also, though he has himself proposed a junctional

theory of phyllotaxis (1913), has also given strong reasons for thinking that whorled phyllotaxis often cannot be explained by junctional factors alone (1936, p. 672; 1938, p. 508). In order therefore to determine more clearly whether this is so, and in order to study the regulating processes if any should be found, it seemed desirable to operate upon apices of species in which such regulating processes were likely to act most strongly. For this purpose species of Labiatae were the obvious choice, since all species of this family have whorled phyllotaxis and nearly all of them have very regular whorls of two, though a few are said to have whorls of higher number (Schoute, 1936, p. 678). The results therefore of diagonal splits and of some other operations on apices of various Labiatae will here be reported and discussed.

As to Schoute's opinions (1938) about the nature of the supposed regulating process, these will be discussed at the end of this paper. But here it seems necessary to answer his criticisms of our own conclusions. He writes (1938, p. 510): 'In these regenerated apices the authors observe that the new leaves are always to be found in those places left free by the edges of the older leaves; consequently they conclude that the position of the new leaves has been determined by these edges': and he goes on to maintain that this argument is unsound, on the grounds (if the writer understands him rightly) that the new leaves may first be determined independently of the spaces between the older leaves and then extend laterally so as to fill up such spaces as are available.

But the argument which he criticizes is not ours. Our argument, which seems clearly expressed (1935, p. 71 seq., and 1931, p. 8 seq.), amounts to this, that in our experiments the *next* leaf always arose in the largest gap between and above the edges of the previous leaves, even when that gap was in a quite abnormal position. Thus the *time sequence* in which the leaves arose in the different gaps followed the order of the sizes of those gaps, and we took this to show that the positions of the leaves depended on the positions of the gaps, and further, to indicate that a leaf arose in each gap as soon as it attained some minimum width and depth (1931, p. 16). The time sequence of the leaves was of course inferred from the levels of their insertions.

Schoute writes also (1938, p. 510) that, 'without any treatment the operated apices . . . would have become spiral in a very short time' on passing into the flowering stage, and he therefore considers it 'very natural' that some of the regenerated apices were spiral. We stated previously (1935, p. 65) our reasons for considering that the spirals in the regenerated apices could not be due to any supposed change to the flowering stage, and there is little that need be added now. The normal apices of *E. hirsutum* begin to form spirals when they pass into the flowering stage, but scarcely ever before they do so; and it is therefore irrelevant that the apices operated upon or the regenerated apices would soon have passed into the flowering stage, since actually of the thirty-seven spiral regenerated apices none did do so during the whole period of experiment (during which they usually formed eight leaves or more), except two (nos. 37*a* and 58) which reached the flowering stage only when they had formed nine and six leaves respectively since the operation.

The level at which an apex passes into the flowering stage can indeed be easily recognized in transverse sections as the level at which axillary flower buds first appear instead of vegetative buds, the flower buds being larger and rounder. Out

of twenty-three normal apices of this species which were pickled about when they were passing into the flowering stage and afterwards sectioned, only one had begun to make a spiral worth the name before the flower buds appeared, having formed four leaves in spiral sequence before the flower buds. In a few others the two or three pairs of leaves of the decussate phyllotaxis just before the flower buds had a very slight spiral tendency, but not enough for the phyllotaxis to be classed as spiral. It may also be noted that the transition to a spiral on flowering is gradual, whereas in the experiments the spirals started suddenly with the first leaves after the operations.

## 2. SPECIES AND METHODS

The diagonal splits to be reported here were made on apices of four species of Labiatae, *Coleus Blumei*, *Stachys tubrifera*, *S. silvatica*, and *Salvia coccinea*. The plants of all the species except *Coleus Blumei* were used when quite small, those of *Stachys tubrifera* being grown from tubers, and those of the last two species from seed. None of the regenerated apices passed into the flowering stage during the periods of the experiments, except two apices regenerated after one split in *Coleus Blumei*, which after the operation made twelve and five vegetative leaves respectively before making floral bracts. *Coleus Blumei* and *Stachys tubrifera* cannot be recommended for such operations, as their apices often died; but with *Stachys silvatica* and *Salvia coccinea* the splits succeeded and gave two regenerated apices almost every time. *S. coccinea* is the more convenient as its internodes elongate later. Other operations which were made on the last three species were the splitting in a vertical radial plane of an  $I_1$  (the region from which one of the next pair of leaf primordia was due to arise), or of a  $P_1$  (one of the youngest pair of primordia visible at the time of operation), and the splitting of a  $P_1$  in a tangential plane. These operations were made mainly to test the relation between leaf and axillary bud, and we have reported the results relevant to that question already (1942).

The method of operating was the same as we have described before (1931, 1935, 1937), except that a binocular giving 52 magnifications instead of 30 was often used with advantage. Subsequently the apical buds were pickled, embedded in collodion, sectioned transversely and drawn under a projector as before. The times before pickling were usually enough for about eight leaf primordia to be formed after the operation. These times varied with the temperature and species.

## 3. THE ORIGINS OF THE PHYLLOTAXIS SYSTEMS AFTER THE SPLITS

The results of the diagonal splits are summarized in Table 1. From this table two main points are evident, first that even in Labiatae the apices regenerated from the halves after diagonal splits sometimes make spirals, and second that they do so much

Table 1. *Phyllotaxis after diagonal splits*

Species	Decussation or approach thereto	Spiral	Irregular and other systems
<i>Stachys tubrifera</i>	17	0	0
<i>Coleus Blumei</i>	9	1	4
<i>Stachys silvatica</i>	13	4	2
<i>Salvia coccinea</i>	2	2	0

less often than in *Epilobium hirsutum* in which species we found (1935) that thirty-seven out of fifty such apices made spirals. One of the spiral apices (*Stachys silvatica*) with eight leaves formed since the operation, is shown in Fig. 1, and another of the same species with nine leaves in Fig. 2. Another (*Coleus*) had formed five leaves, and the remaining four (two *Stachys silvatica* and two *Salvia coccinea*) did not continue spiral but returned to whorls of two after making five, four, four and three leaves in spiral sequence. Three of these are shown in Figs. 3-5, and their returns to whorls will be discussed in § 4.

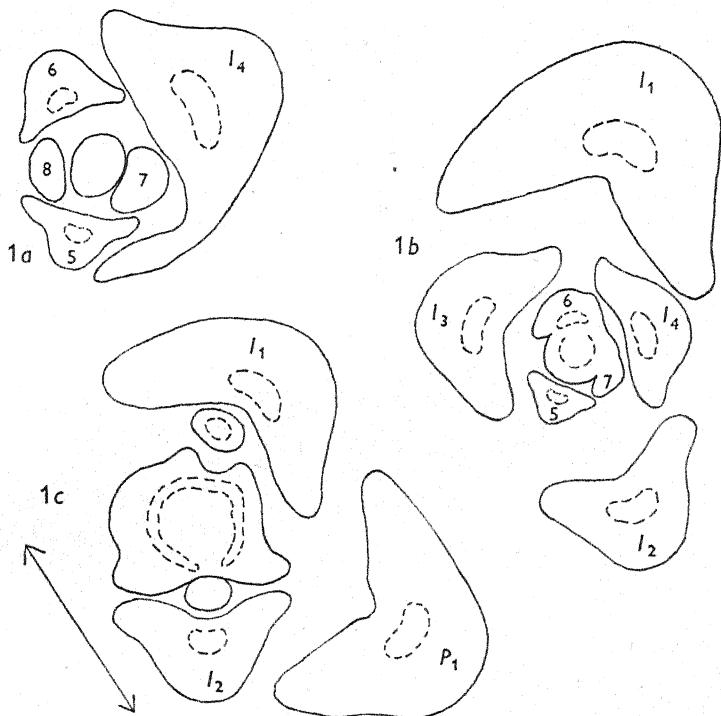


Fig. 1 *a, b, c. Stachys silvatica*. A spiral apex regenerated after a split. *a, b, c* are transverse sections at different levels from above downwards. The plane of the split is shown approximately in *c* by a double-headed arrow. The outer leaves are omitted from *a*. *a*  $\times 53$ , *b*  $\times 31$ , *c*  $\times 23$ .

We have already interpreted the origin of spirals after diagonal splits on a 'junctional' basis (1935, p. 68). In brief, the split allows more room on one side of  $P_1$  than on the other, and consequently the next two leaves that arise after the operation ( $I_1$  and  $I_2$ ) arise in succession and at different levels. Also these two leaves are usually not quite opposite, and usually converge towards the wound, though sometimes they diverge from it. Thus the system is asymmetric from the start and spiral phyllotaxis is to be expected.

The question arises therefore, How is it that in the Labiatae the regenerated apices were so seldom spiral and were much more often nearly decussate or became so? One first step towards decussation was that in the Labiatae, as Table 2 shows, the first two leaves that arose after the split were often in a whorl together, being practically at the same level, although they were seldom exactly opposite, whereas



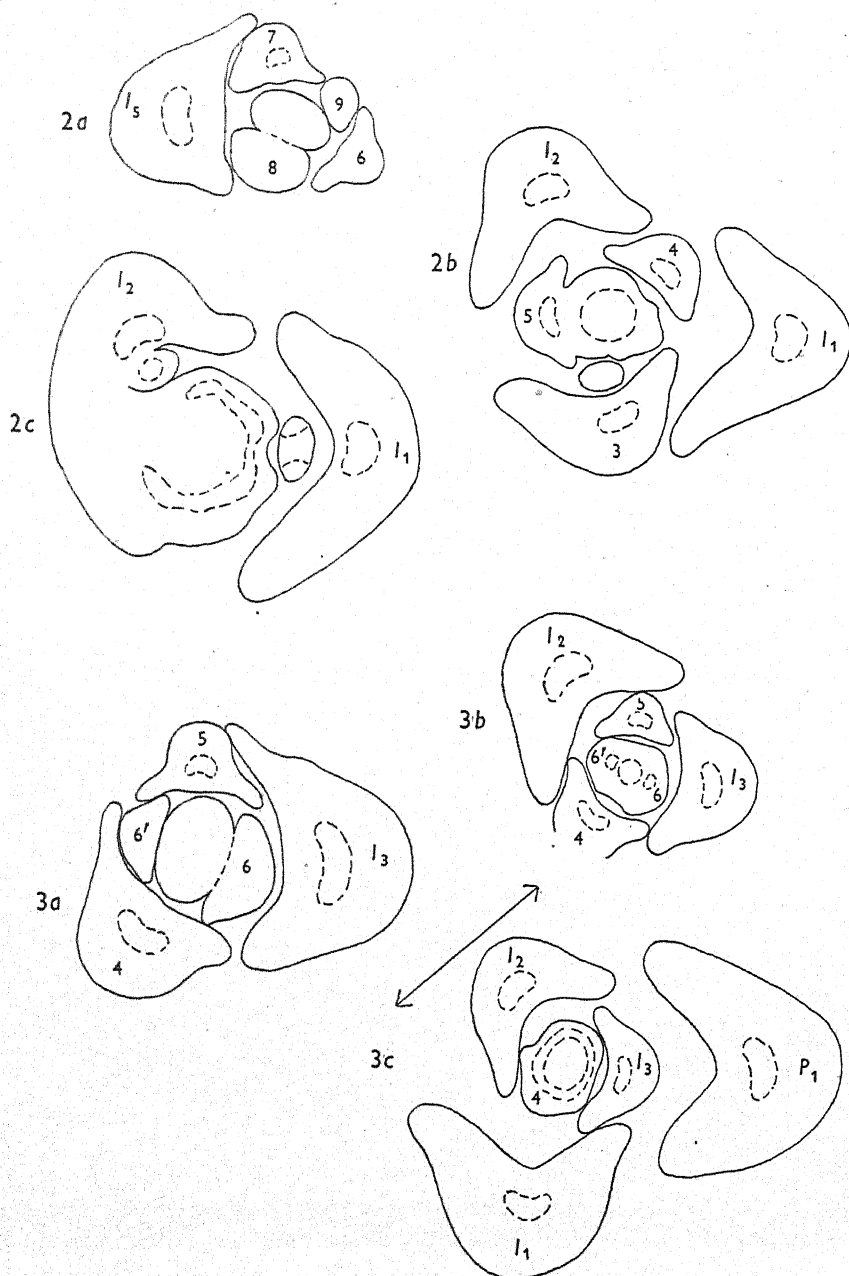
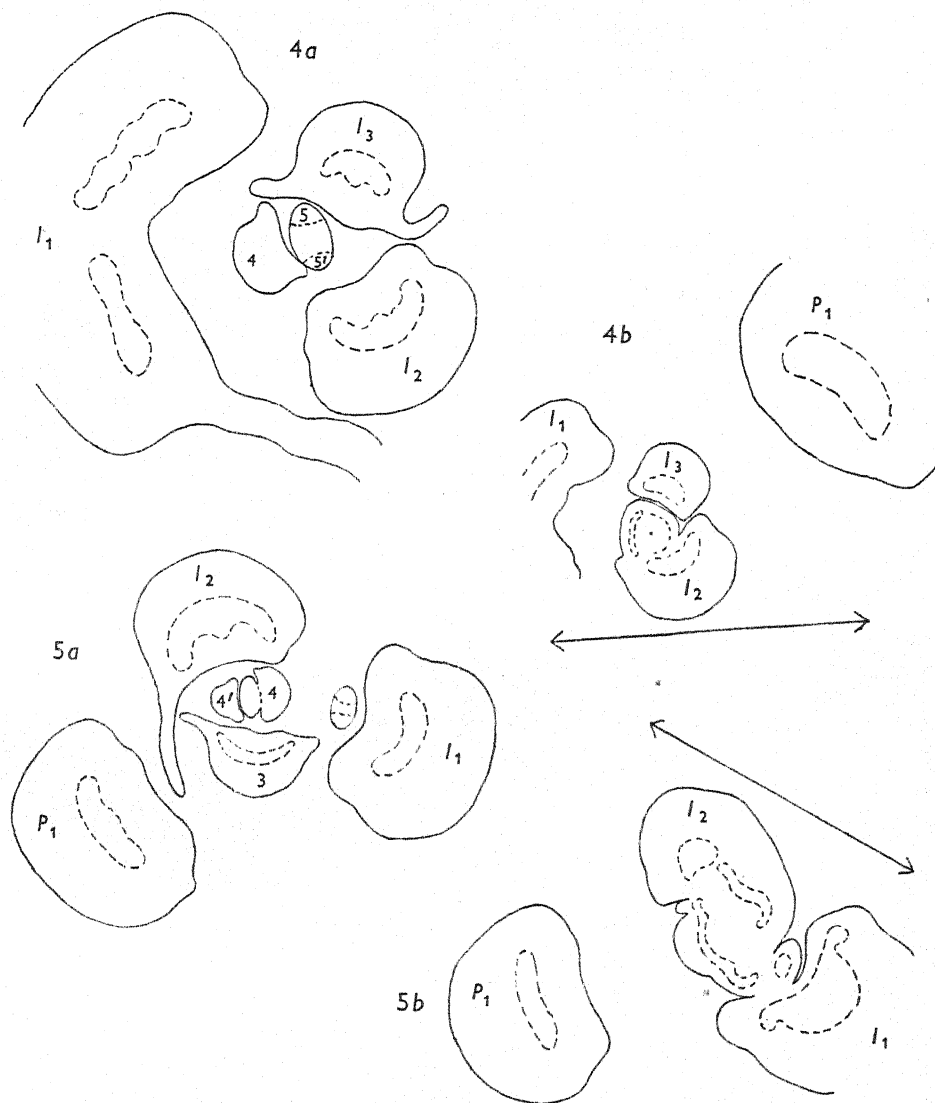


Fig. 2 a, b, c. *Stachys silvatica*. Another spiral regenerated apex.  $a \times 53$ ,  $b$  and  $c \times 31$ .

Fig. 3 a, b, c. *Stachys silvatica*. A spiral apex which returned to a whorl of two.  $a \times 53$ ,  $b \times 31$ ,  $c \times 23$ .



Figs. 4 a, b, and 5 a, b. *Salvia coccinea*. Two spiral apices, regenerated after one split, which returned to whorls of two. 4 a  $\times 53$ , 4 b and 5 a, b  $\times 31$ .

Table 2. The level of insertion of the next leaf ( $I_1$ ) or the next two leaves after a split

Species	$I_1$ not in a whorl	Two $I_1$ 's in a whorl together	$I_1$ in a whorl with $P_1$	Not noted
<i>Stachys tuberifera</i>	0	10	7	0
<i>Coleus Blumei</i>	3	7	0	4
<i>Stachys silvatica</i>	9	10	0	0
<i>Salvia coccinea</i>	2	2	0	0

in *Epilobium hirsutum* these two leaves were *never* at the same level, and nearly always quite far from being so.

This different reaction of the Labiatae already suggests that there works in them some kind of 'whorl-making' factor in addition to the junctional factors. But since the junctional conditions immediately after the split are difficult to determine very exactly, it will be more profitable to study in detail the evidence for such a factor when it can be found at a later stage; and this will be done in § 4. For since the next two leaves after the operation were seldom opposite, even when at the same level, some further regularizing step was needed to make the system return to something approaching a symmetric decussation as it so often did. Also four of the apices in which  $I_1$  was not in a whorl returned nevertheless nearly to decussation (one *Coleus* and three *Stachys silvatica*), and these also will be discussed in § 4. Of the remaining apices in which  $I_1$  was not in a whorl, seven formed spirals and two formed irregular or other systems.

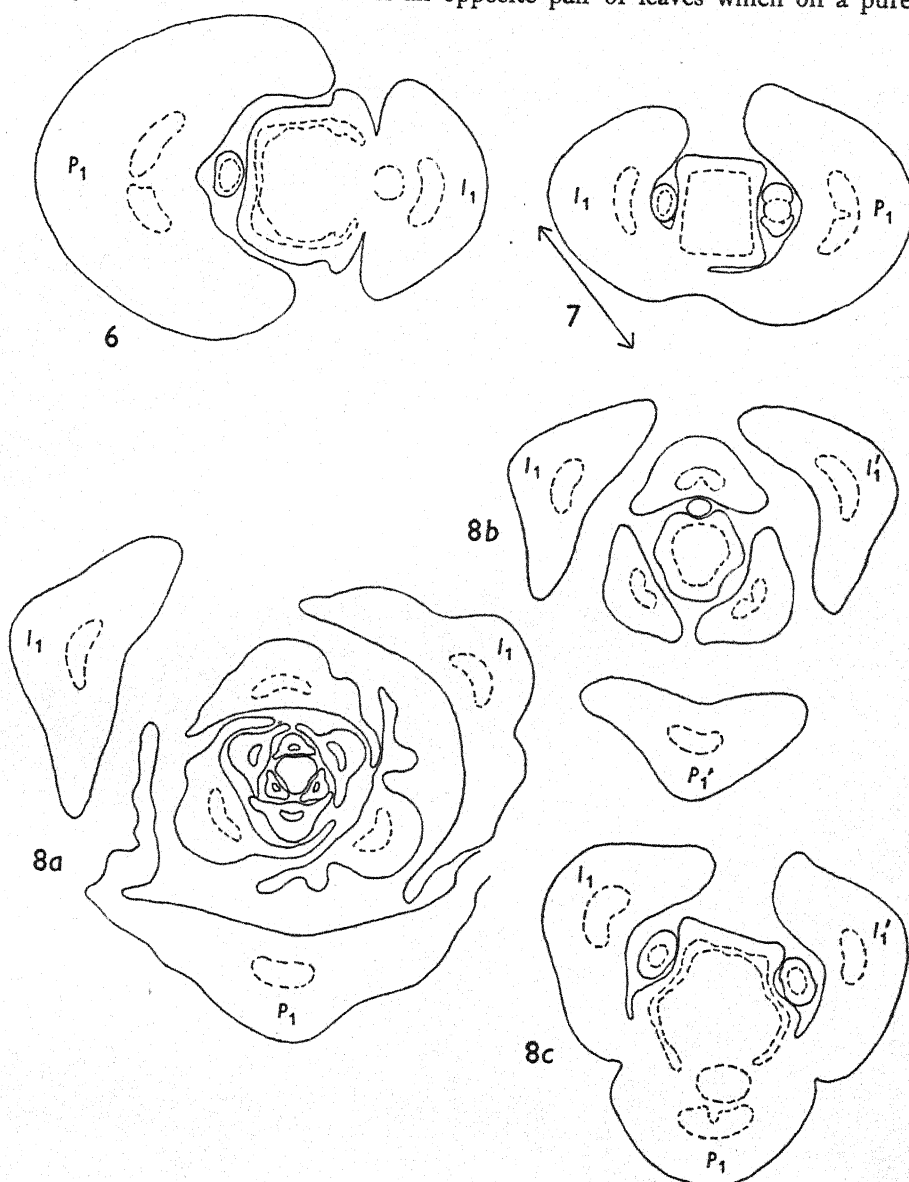
Table 2 shows also that in seven apices of *Stachys tuberosa*  $I_1$  formed a whorl with  $P_1$ . Examples are shown in Figs. 6 and 7. This was a very surprising result, but it can hardly be a mistake, since  $P_1$  could be identified by counting up from an older leaf marked at the time of operation, and also since it was noted seven times, but only in the one species. Tables 1 and 2 indicate that the whorl-making factor is strongest in *S. tuberosa*, less strong in *Coleus* and less strong again in the other two species.

Here may be mentioned also amongst the apices operated upon in other ways one apex (*Stachys tuberosa*) which formed a very good system of whorls of three (Fig. 8). In this apex a  $P_1$  had been split tangentially and had not developed. The two  $I_1$ 's are almost in a whorl with the opposite intact  $P_1$ , and subsequent whorls alternate regularly.

#### 4. THE RETURNS TO DECUSSATION

We described previously (1935, p. 79 and Fig. 12) a bilaterally symmetric oscillating phyllotaxis which was sometimes found after operations on *Epilobium hirsutum*. In this system a pair of leaves that are at the same level but not opposite is followed by a second pair that are opposite but not at the same level; and these are followed in turn by a third pair which, like the first, are at the same level. These converge towards the lower leaf of the second pair and so in the opposite direction to the first pair. On a purely junctional theory this system can readily be understood and should continue indefinitely, as it often did in *Epilobium*. In many of the present experiments a beginning was provided for this system when the  $I_1$ 's were at the same level but not opposite, and in others of these experiments beginnings were provided for it in various other ways; but then the system instead of continuing usually returned to decussation or nearly to it. Since it does not seem possible to explain these returns to decussation by junctional factors alone, they indicate that some regularizing factors are at work in addition to the junctional factors. They also show very clearly the different ways in which these factors can work, since some of these returns to decussation took place at the first of the two phases through which the oscillating system passes and some at the second. Sometimes again the return was more gradual, and then it was less instructive.

The first way in which the oscillating system returned to decussation was by causing to arise at the same level an opposite pair of leaves which on a purely



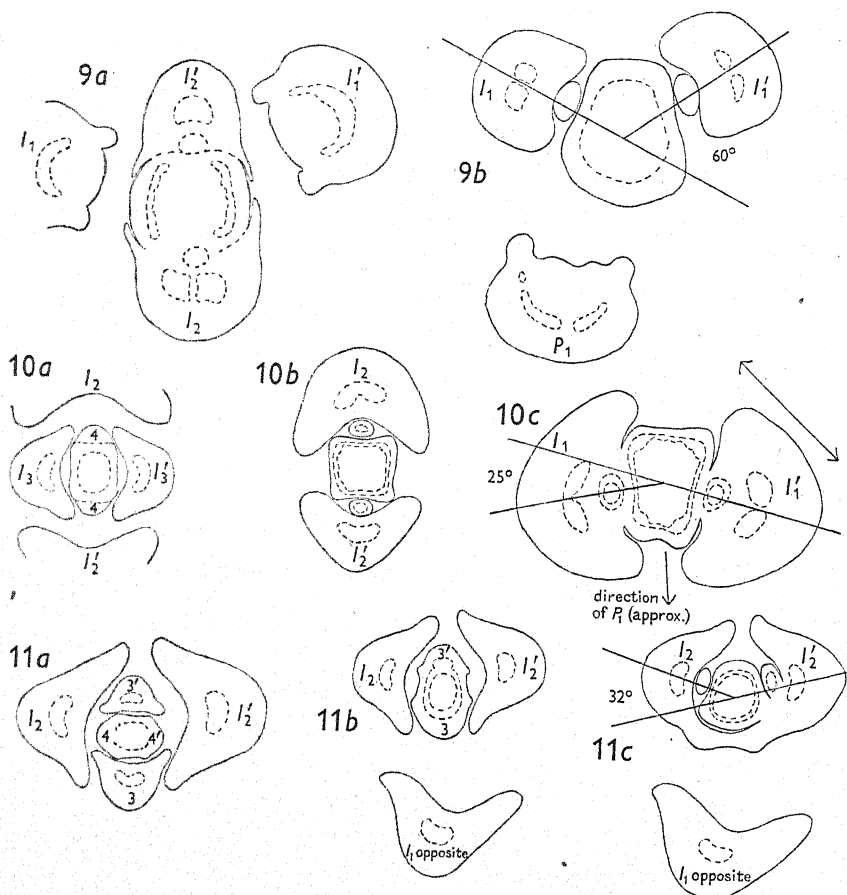
Figs. 6, 7. *Stachys tuberosa*. Sections of two regenerated apices in which  $P_1$  and  $I_1$  were in a whorl together. Both  $\times 21$ .

Fig. 8 a, b, c. *Stachys tuberosa*. An apex which formed whorls of three, when one of the  $P_1$ 's had been split tangentially and had failed to develop. a  $\times 21$ , b and c  $\times 16$ .

junctional theory should have arisen at different levels, since they followed a pair which was not opposite. They were, however, unequal in size, as would be expected. Examples are shown in Figs. 9 (*Coleus*), 10 (*Stachys tuberosa*) and 11



(*S. silvatica*). In these examples a pair of leaves which arose after the operation were far from being opposite, but yet the next pair were practically at the same level, and consequently the next pair again were practically opposite. The first two of these apices had been regenerated after diagonal splits, and in the third apex an  $I_1$  had been split radially and had not developed. Altogether eleven very clear examples



Figs. 9 a, b, *Coleus Blumei* and 10 a, b, c, *Stachys tuberifera*. Two apices regenerated after splits which returned to regular decussation by means of a pair of leaves at the same level following a pair which were not opposite. All  $\times 16$ .

Fig. 11 a, b, c. *Stachys silvatica*. An apex which returned to decussation in the same way when the presumptive area of one of the  $I_1$ 's had been split radially, and the  $I_1$  had failed to develop. a  $\times 21$ , b and c  $\times 16$ .

of this kind of return to decussation were found amongst the Labiatae. The angles by which the first pair of leaves deviated from being opposite ranged from 21 to 60°.

Similar returns towards decussation were often found in *Epilobium hirsutum* after isolations of a  $P_1$ . For often the  $I_1$ 's converged strongly towards the wound, but yet the  $I_2$ 's were at practically the same level though unequal, and the  $I_3$ 's nearly opposite. We thought previously (1935, p. 81) that these returns were due to the much larger size of one of the  $I_2$ 's, but it now seems to the writer more

probable that the primary factor was the bringing of the  $I_2$ 's to nearly the same level, in most of these apices at least. The previous explanation may, however, still hold good for a return to decussation after a split illustrated previously (1935, Fig. 10, p. 81), which was not preceded by an adjustment of levels. In any case the *Epilobium* apices did not always return to decussation after an isolation of  $P_1$ , but sometimes continued to oscillate, as also they did after some of the diagonal splits (1935, Fig. 12 and p. 79).

The second way of returning to decussation was by causing to arise in practically opposite angular positions a pair of leaves which on a purely junctional theory should not have been opposite, since they followed a pair which were at different levels. Six examples were found, all of them in *Coleus* after diagonal splits, and one of them is shown in Fig. 12. In this apex the beginning of the system after the operation was not identified, so the pairs of leaves are given arbitrary numbers. The leaves 1 and 1' are far from opposite and 2 and 2' are consequently at very different levels, the lower leaf being in the larger gap, since apparently no regularizing factor had intervened at this stage. But leaves 3 and 3', instead of converging towards the lower leaf of the previous pair are practically opposite, and the phyllotaxis is finally regular.

What may be a variation of this second way of returning to or towards decussation was found in four apices regenerated after diagonal splits (three *Stachys silvatica* and one *Coleus*), in which there was a single  $I_1$  in a roughly transverse position and then a pair of  $I_2$ 's which were not far from being opposite. One of these (*Coleus*) is shown in Fig. 13. But the interpretation of these four apices is less certain, since the region of the apex opposite to  $I_1$  and on a level with it, where another leaf might have been expected, was close above the wound, and it is difficult to be sure exactly what were the junctional conditions in this region after the operation.

Still a third kind of return to decussation was a return made directly from a spiral. This was found in four apices regenerated after splits (two *Stachys silvatica* and two *Salvia coccinea*). Three of these apices, which were very striking and were shown in Figs. 3-5, made a pair of opposite leaves at the same level following directly upon a number of leaves in spiral sequence, so that the levels and angular positions of this pair of leaves must both have been regulated at once. In the fourth apex the return was more gradual.

##### 5. CONCLUSIONS FROM EXPERIMENTS

The experiments reported show that in the species of Labiatae the positions of the new leaf primordia are often regulated by some causal factors which are distinct from the 'junctional' factors—distinct, that is, from the spatial conditions set up by the older leaves or other contact members just below. For two leaf primordia which on a purely junctional theory would be expected to arise at different levels may be somehow caused to arise at the same level, and two leaf primordia which would not be expected to be opposite in angular position may be somehow caused to be opposite: or again the same two leaf primordia may be adjusted in both respects at once. These regulating factors, which apparently are peculiar to species with whorled phyllotaxis, should be considered as acting not instead of the junc-

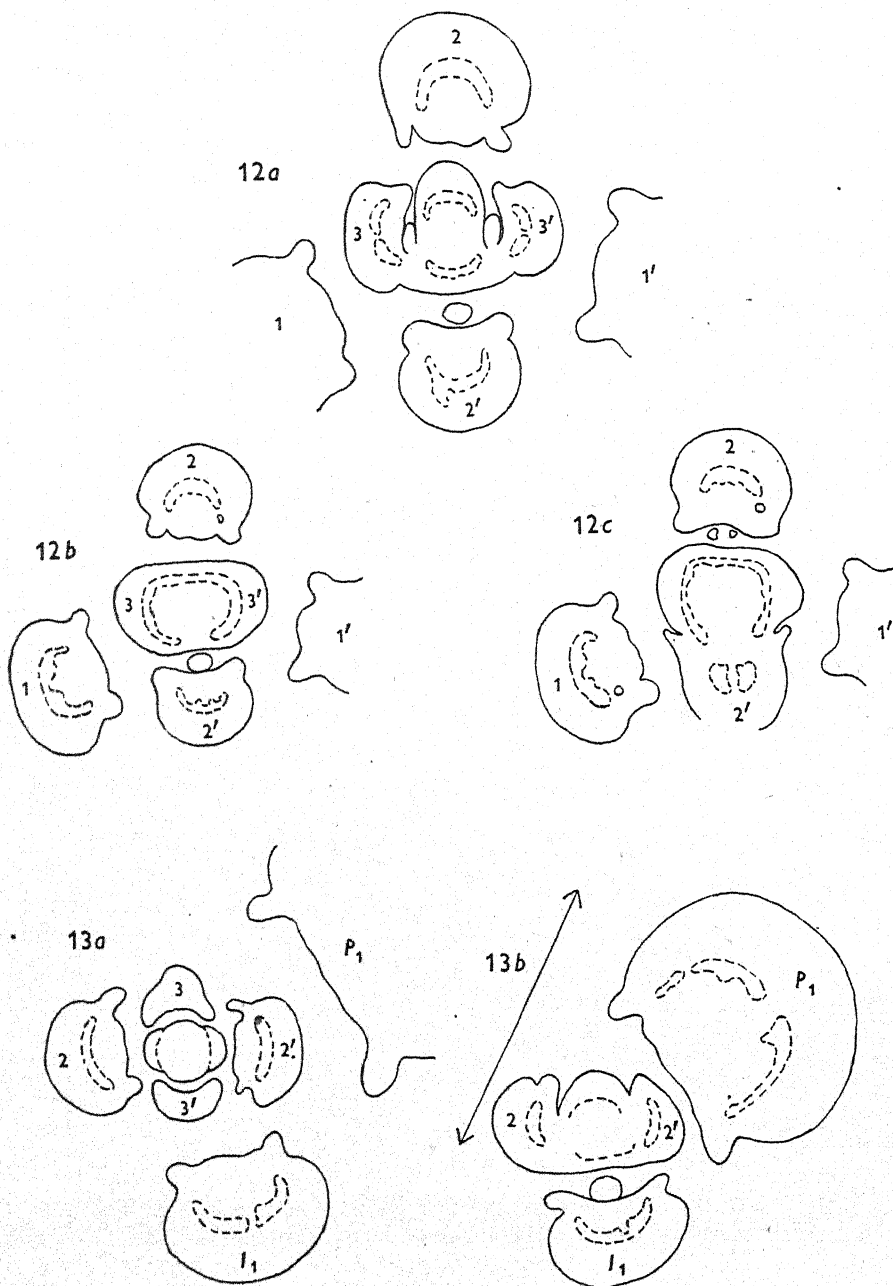


Fig. 12 a, b, c. *Coleus Blumei*. A regenerated apex which returned nearly to decussation by means of a pair of opposite leaves following a pair which were not at the same level.  $a \times 23$ ,  $b$  and  $c \times 18$ .

Fig. 13 a, b. *Coleus Blumei*. A regenerated apex which formed a nearly opposite pair of leaves following a single leaf.  $a \times 31$ ,  $b \times 23$ .

tional factors but in combination with them. For sometimes the regulating factors fail to act when they might be expected to act, and then the effects of the junctional factors are clearly revealed.

Thus in the spiral apices, so long as the spirals continued, the positions in which the leaves arose were in accordance with the junctional factors alone, though finally some of the spirals returned to decussation. Again in Fig. 12, as already noted, the different levels of leaves 2 and 2' are in accordance with the junctional effects of the leaves below them and have not been regulated towards decussation, although the system had already returned to bilateral symmetry. In a few other apices also junctional factors were found to have acted without being interfered with by any regulation. It is certainly puzzling that the regulating factors sometimes failed to act in this apparently capricious way.

In *Epilobium hirsutum* there seems to be at least one of these regulating factors at work, the one which tends to equalize the levels of the insertions of a pair of leaf primordia, though the regulation is much weaker than in the Labiatae. So at least it now seems to the writer after consideration of the results of the isolations of  $P_1$  in *Epilobium* and after comparison with the experiments on the Labiatae, though previously we considered that such a conclusion would be premature (1935, p. 93). That the regulation is, however, weaker in *Epilobium* than in the Labiatae follows from the much greater frequency of spirals after diagonal splits, and from the fact that in *Epilobium* the oscillating bilateral system described in §4 and some other slightly irregular systems much more often continued their irregularities through several stages in ways that can readily be understood on a junctional theory.

With regard to the second regulating factor which in the species of Labiatae sometimes makes two leaves arise opposite to each other when without it they would not have done so, an interesting point arises. For one of these species was able to make a very good system of alternating whorls of three after an operation (Fig. 8), and according to Schoute (1936, p. 678) probably all decussate species sometimes make whorls of three. This makes it appear that the second regulating factor is not one which tends to make two leaves of a whorl arise opposite to each other, but one which tends to equalize all the divergence angles in a whorl, whether they are two or more than two. There are therefore good grounds for considering that the regulating factors which adjust the levels and the horizontal angles are distinct, and are not merely two aspects of a single tendency to form regular whorls of two: for the decussate species do not seem to have exactly that tendency. The fact that these species normally have only two leaves in a whorl is probably due to junctional factors. As to how the regulating factors produce their effects, there is at present no adequate evidence.

I am indebted to my wife for performing more than half of the operations of which the results are here reported.

#### 6. SCHOUTE'S THEORIES OF WHORL FORMATION

Schoute (1922, 1925, 1936, 1938) has reached conclusions concerning whorled phyllotaxis of which some must here be discussed. He has reported many interesting observations, though unfortunately they were all made on grown shoots, not on



bud sections, and his papers, especially the most recent one (1938), contain also many valuable and learned accounts and discussions of the opinions of other workers. They should be consulted for references. Schoute distinguishes between what he calls 'true' and 'false' whorls (1936, pp. 670, 672; 1938, p. 500). The distinction which he draws amounts in effect to this, that 'true' whorls, which he considers to be rare, unstable and transient, are those which can be explained by junctional factors alone, and 'false' whorls are those which cannot. This being so, it would surely be better to call them junctional and non-junctional whorls: for plants make no statements, so why should they be accused of falsehood? Amongst the non-junctional or 'false' whorls Schoute further distinguishes between what he calls 'growth' whorls and 'binding' whorls. As examples of 'growth' whorls he describes (1922) some lilies and umbellifers and a few species of other families, and concludes that their leaves arise in spiral sequence, but afterwards become associated into something approaching whorls through uneven growth of the stem, which elongates in some zones and not in others. Nearly all other whorls he classifies as 'binding' whorls, and since this class, in which the whorls are usually quite exact, includes the whorls of Labiatae and other families with decussate phyllotaxis (1936, p. 678; 1938, p. 416), it is his theory of 'binding' whorls which must here be discussed. This theory is the following. The leaves are supposed to be determined in spiral sequence, and this Schoute considers to be due to junctional factors (1936, p. 672), having himself previously proposed a junctional theory of phyllotaxis (1913). But later the leaves which are going to form the separate whorls become connected or bound together into separate cycles, either after or even before they become visible (1925, p. 129) by some process of unknown nature taking place within the stem (1938, p. 416); and when the stem elongates these leaves are held together at the same level by the binding. Furthermore, since whorls of two leaves, and some whorls of higher numbers, are usually quite regular even when the leaves first arise, so far as the eye can judge, and alternate regularly, Schoute postulates three other regulating processes: that the divergence angles between the leaves are made equal through displacements of the leaves which occur after or even before they arise and are due to some process of 'almost unknown nature' (1938, p. 434), that the sizes of the leaves of a whorl are somehow made equal (p. 446), and that the whorls are somehow made to alternate regularly (p. 444). Thus the binding is supposed to account only for one out of four regulating processes in the whorls named after it.

Another prominent feature of Schoute's theory is that a spiral sequence of leaves is supposed to be present in principle, though invisible, and to run right through the whorled phyllotaxis. He claims to find indirect evidence for this spiral in various secondary phenomena, and from the spiral the whorls are supposed to be formed by the factors mentioned above. This part of his theory seems to the writer very difficult to maintain. For the supposed spiral sequence is clearly considered by Schoute to be due to junctional factors (1936, p. 672) in accordance with his original theory of phyllotaxis (1913). Let us then consider a plant of a species with whorled phyllotaxis, for simplicity one with decussate phyllotaxis in which two leaves are about to arise. According to Schoute these two leaves would arise in spiral sequence, were it not that the whorl-forming factors intervene and cause them to arise at the same level and opposite, and to be of equal size. Very well then,

when these two leaves have arisen in this way, it is to be expected *on junctional grounds alone* that the next two leaves after them will arise as regular members of another whorl, alternating with the previous whorl of two: so how can any hidden spiral sequence based on junctional factors continue any longer?

The hidden spiral could continue only if the position of a leaf were determined much earlier—at about the  $I_6$  stage if the leaves are to be numbered singly along the supposed spiral. For then the leaves  $I_3$ ,  $I_4$  and  $I_5$  would be those of the cycle just below, of which the positions would determine the position of  $I_6$ ; and they themselves might not yet have been shifted into the regular whorled positions by Schoute's regulating factors. But it is most improbable that leaves are determined anything like so early as this, since the evidence of many of our experiments, including many on decussate species, is quite opposed to it. Thus many of our experiments indicate that in decussate species even the  $I$ 's are often scarcely determined, since their positions are often shifted by operations made not on them but on the  $P_1$ 's (see, for example, Fig. 8).

As to the numerous observations which Schoute offers as indirect evidence for a hidden spiral (1938), they do not seem to the writer decisive, though many of them are certainly interesting in other ways: but unfortunately it is not possible to discuss them briefly. In some species of *Peperomia* indeed, which appear to have whorls of leaves on the developed shoot, Schoute may have succeeded in tracing a spiral along the shoot (1925). But he does not report that he has looked to see whether in these species the leaves are in whorls when they arise. Very probably they are not, for Goebel (1913) has drawn sections of buds of other species of *Peperomia* which are apparently whorled lower down the shoot, and in these the leaves appear to arise in rather irregular spiral sequences—certainly not in whorls. So from the observations on these plants it cannot be concluded that there is a hidden spiral in plants of other families, in which the leaves *arise* in regular whorls.

But although Schoute's theory of a hidden spiral in regularly whorled species seems untenable, it remains possible that these species may be able to regulate their phyllotaxis by equalizing the levels at which the leaves arise and the divergence angles between them, as Schoute believes that they can. Indeed, it has been shown in the present paper that species of Labiatae often do regulate their phyllotaxis in these ways after it has been disturbed by an operation, and presumably they often do so after other disturbances also, whether these are due to external or internal causes. So it must next be considered whether the equalization of the levels of leaves in these species may be due to binding, in some such way as Schoute proposes, and the argument by which he supports this idea must be examined. His argument is based on the well-known phenomenon of biastrepsis or forced torsion (1936, p. 674; 1938, p. 417). Biastreptic plants have violently twisted stems, and it is agreed that they are plants in which exceptionally the leaves have arisen in spiral sequence, though the plants belong to species which normally have whorled phyllotaxis. An explanation was offered in 1854 by Braun, quoted by De Vries (1892*a*, p. 332), and was supported by De Vries (1892*a*, *b*). It is that in normal plants of these species the leaves of each whorl are somehow bound together, but the binding does not impede the growth of the stem. When, however, from unknown causes a plant of one of these species makes its leaves in spiral sequence, then the

binding continues without interruption, usually along the genetic spiral, and so forms a continuous spiral band. Consequently when the stem elongates the resistance of this band leads to a tangential force which twists the stem in the opposite direction to that of the spiral. The stem does, however, succeed in elongating, though less than is normal.

Schoute's explanation (1936, p. 674) is a little different. For since he believes that even in the normal plants of these whorled species the leaves are determined in spiral sequence, he is led to conclude that the primary difference in the biastreptic plants is that in them the binding proceeds continuously along a spiral (usually the genetic spiral), whereas in the normal plants it works intermittently, binding into a cycle or ring the leaves which are going to form a whorl and ceasing between the whorls. He thus believes that the binding which leads to biastrepis is the same process as that which makes whorls (1938, p. 417), but differently directed. Common to both explanations is the conclusion that the cause of biastrepis is the binding into a spiral band of leaves which arise in spiral sequence, however that binding may originate.

Now the direct evidence for this conclusion is provided by some experiments of De Vries (1892*a*, p. 335; 1892*b*, p. 408) who interrupted the biastreptic binding by making longitudinal cuts in the stem, and found afterwards that at the levels of these cuts no torsion developed. Since he made the cuts at a level a little way at least below the apical bud, the biastreptic binding had not yet produced its effects above this level, and there are no grounds for thinking that any similar process takes place close to the stem apex and even above the youngest visible leaves, where on Schoute's theory the binding must be supposed to act in regularly whorled species. At the most the biastreptic binding, so far as it is at present demonstrated, could only provide a *comparison* tending to support Schoute's theory.

But does it even do this? In biastreptic plants the stem does succeed in elongating and so increases the differences between the levels of successive leaves in spite of the binding. In the normal whorled plants the binding might be more effective, since the leaves might, as on Schoute's theory, be bound together in closed rings or cycles instead of a continuous spiral band. But still one would expect from comparison with biastrepis that if from any cause the leaves in a cycle were determined at rather different levels, the stem or stem apex in that region would still be able to elongate at least a little and so to increase the differences of level between the leaves of the cycle. In so far, therefore, as comparison with biastrepis is valid at all, it tells rather against Schoute's theory.

So there seems to be no published evidence indicating that equalization of levels is caused by binding, though naturally it *may* be so caused in some instances at least. But in any case it seems unlikely that it can be so caused even before the leaves arise, as Schoute believes. It may also be asked how it is, if binding prevents two leaves of a pair from becoming separated vertically, that it does not also prevent them from becoming separated horizontally, when the angle between them is increased to  $180^\circ$  by the second regulating factor.

Finally, it may be useful to compare with Schoute's hypothesis the conclusions concerning regulating factors in whorled phyllotaxis to which the present results

have led. The present evidence shows only that in the Labiatae these factors of unknown nature, one which tends to equalize the levels of neighbouring leaves and one which tends to equalize the angles between them, intervene often, though not always, so as to restore towards regularity a whorled system which has been upset by an operational cut. Presumably, therefore, they intervene so as to regularize whorled systems which have been upset in other ways also, but there is no reason for thinking that they act all the time. For when such a system is regularized, it is to be expected on junctional grounds alone that it will continue regularly, so long as no change occurs in any essential factor external or internal to upset it.

As to Schoute's third hypothesis, that there is a regulating factor which equalizes the sizes of the leaves of a whorl, this is not necessary, except to support his theory, nor is it true. For when the junctional conditions are such that the leaves of a whorl would not be expected to be equal in size, they actually are not equal, as is clear from the eleven examples mentioned in § 4, of which three are shown in Figs. 9-11. The fourth factor regulating the alternation of whorls, which he postulates, is also unnecessary except to support his theory.

#### 7. SUMMARY

1. When stem apices of species of Labiatae are split longitudinally in a diagonal plane of the phyllotaxis, which is decussate (with alternating whorls of two), the apices regenerated from the halves sometimes form spiral phyllotaxis, but much more often they are or become decussate again or nearly so.

2. The ways in which the apices return to decussation after the splits are described and discussed, and it is concluded that after a disturbance the apices of the Labiatae can regulate their phyllotaxis towards decussation by means of two regulating factors which are of unknown nature and are presumably peculiar to whorled species—one which tends to equalize the levels at which leaves arise, thus forming whorls, and another which tends to equalize the divergence angles between the positions in which leaves of a whorl arise.

3. Evidence is also offered showing that the regulating factors of the Labiatae act in combination with the 'junctional' factors, by which term is meant the positions, shapes and sizes of the leaves or other contact members immediately below the zone of the stem apex within which new leaves are being determined. In several instances the effects of the junctional factors were clearly revealed.

4. After previous diagonal splits of decussate apices of *Epilobium hirsutum* we found spiral regenerated apices much more often (1935). In that species the apices have much less tendency to regulate their phyllotaxis towards decussation, and the junctional factors are correspondingly more effective. But it now seems to the writer probable that the first of the two regulating factors can work in that species also, though less strongly.

5. Some criticisms by Schoute (1938) of our previous conclusions (1935) are answered in § 1, and his own theory of the formation of whorls by binding is discussed and criticized in § 6.



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## A TWIN SEEDLING IN *ZEA MAYS* L. TWINNING IN THE GRAMINEAE

By B. C. SHARMAN

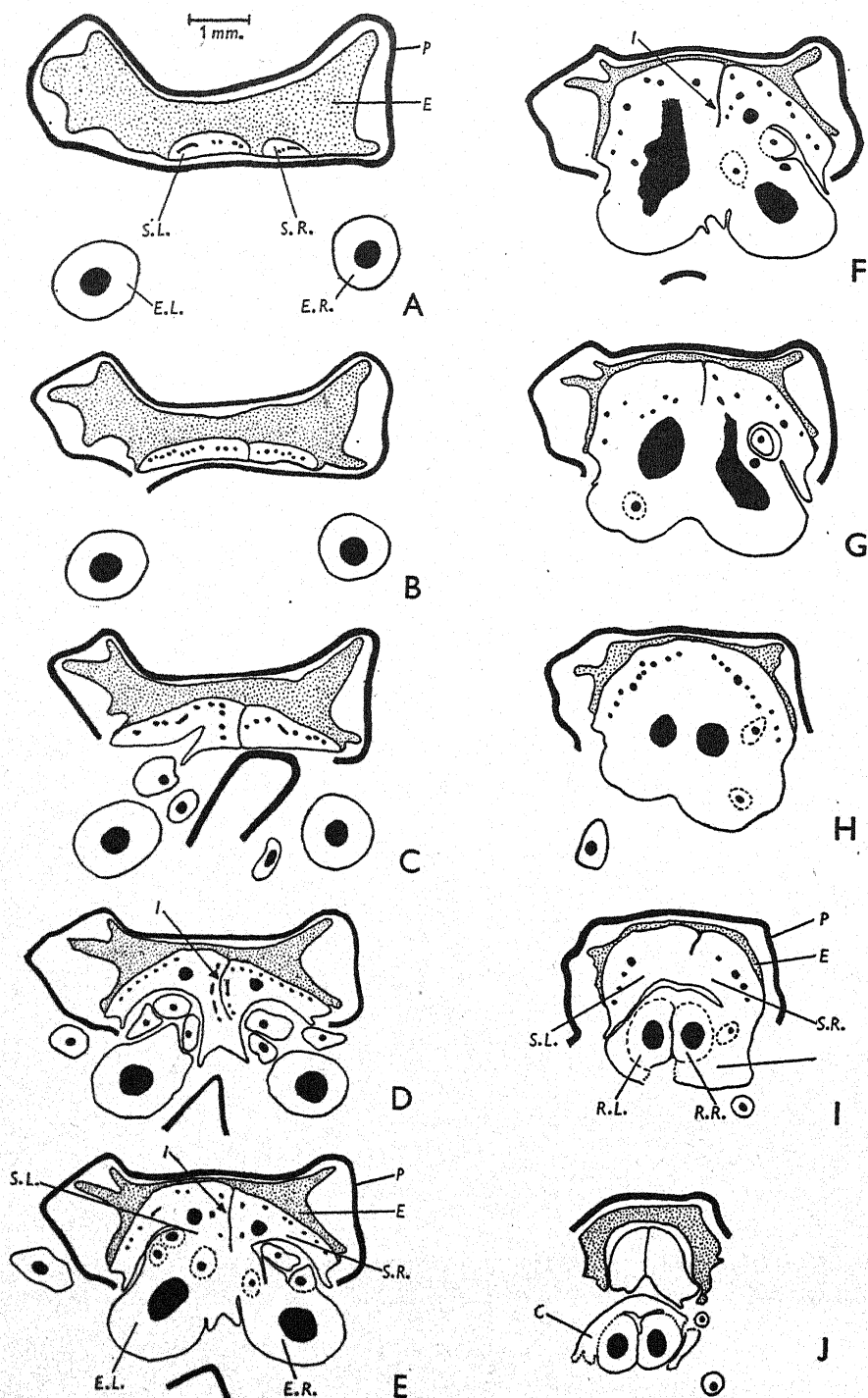
*Department of Botany, University of Leeds*

(With Plate 1 and 1 figure in the text)

Amongst some maize seedlings grown for class use, one was observed to have two embryos emerging from a single caryopsis (Pl. 1 A, B). Because its appearance immediately caused speculation as to the exact relationship of the embryos to each other, it was pickled and sectioned (Text-fig. 1 A-J).

The two embryos appear to be completely separate except for the scutellum, which offers an interesting condition. At the top and bottom (Text-fig. 1 A, B, C and J) it is completely separated into two parts, each being quite normal in appearance. In the region of the union of the epicotyl (Text-fig. 1 D, E, F and G) it is entire but indented, the epidermis being infolded to a considerable degree. At one point (Text-fig. 1 H) there is absolutely no sign of any bisection. From the section illustrated in J in Text-fig. 1, it appears that both radicles were originally enclosed in a single coleorhiza. Although the right-hand embryo is placed a little lower in the caryopsis (Pl. 1 A, and Text-fig. 1 A, E and G) the two appear to have been amazingly evenly balanced in their development and subsequent germination, and to have proved very well-matched rivals for the food supplies. Even as late in development as when photographed (Pl. 1 A) they were still practically mirror images. This even development, the way in which the scutellum seems to have arisen as an essentially single unit and the obvious singleness of the endosperm, testa and pericarp, suggest that the twinning arose early in the life history, probably being caused by longitudinal division or constriction of the more or less spherical mass of cells constituting the 'pro-embryo' stage of Souèges (1924).

There appears to be only a very limited literature bearing on the structure of twin seedlings in grasses. Kempton (1913) deals with a number of maize caryopses which have obviously arisen from two ovaries showing various degrees of 'fusion'. In extreme cases he shows examples of caryopses bearing embryos 'back to back' so that a germ appears on both sides of the grain. These have come from a single spikelet having two flowers, one of which is normally aborted but can, as on this occasion, be fertile. He thinks of his grains as resulting from the fusion of the two ovaries after fertilization, but his illustrations, even of the mature caryopses, would suggest that the union, if such it ever is, has arisen much earlier. He concluded, however, that 'the development of the two ovaries in one spikelet must be simultaneous, as a large number of cases have been found where the two seeds from one spikelet have grown together with a single pericarp. These connate seeds had been fertilized through a double silk which was attached to the pericarp near the union of the two seeds. Connate seeds are a distinct phenomenon from single seeds with



Text-fig. 1. Transverse sections through the maize twin shown in Pl. 1 A. A, above the union of the two embryos; J, just below the departure of the two radicles. C, coleorhiza; E, endosperm; I, indentation of scutellum; P, pericarp; E.L., E.R. epicotyls of left and right embryos; S.L., S.R. left and right portions of the scutellum; R.L., R.R. radicles of left and right embryos.

a double embryo, two of which have been seen.' One of his photographs shows a caryopsis with two embryos growing out side by side in the same manner as the seedling described above. His example, however, is very deformed (probably through the way the caryopsis was lying when it germinated), and while left in the greenhouse to develop further, was eaten by a pest before it could be properly examined.

Blaringhem (1920) described a strain of maize (called by him *Zea Mays* var. *polysperma*) in which many double and sometimes triple grains developed. Stratton (1923) examined this variety developmentally and showed that the double grains arose from two 'coalesced' flowers, the two flowers of the spikelet being fertile and developing, from the inception, in various degrees of 'fusion'. She describes connate and semi-connate grains, the former being kernels with the two embryos on opposite sides of the grain, arranged back to back and enclosed in a common pericarp formed from the coalesced ovaries of the two flowers. She states that in all the cases she examined the connate grains possessed two stigmas, which, however, were often close together and could only be seen as two entities with the aid of a lens. The semi-connate grains showed various degrees of fusion and were 'caryopses with the two seeds less coalesced than in the connate type, the pericarp extending more or less between them'.

Jenkin (1931), when discussing grass breeding, remarks that twin seedlings occur frequently, and warns the breeder to watch for them, since the two plants arising have different characters; this suggests that he is dealing with a different type of twin from that described for *Zea*.

Bledsoe (1929) describes double and triple kernels in florets of rye and wheat crosses, but in all cases his appear to be multiple caryopses arising from multiple ovaries, some of which are shown from dissections of flowers.

Nishimura (1922-3) states that in *Poa pratensis* and *Agrostis alba* numerous examples were found 'where two embryos appeared. These embryos were more commonly placed side by side but in some instances one of the embryos would occupy an oblique position to the median plane of the grain.' He also observed a number of triplets in *Poa pratensis*, one of which he illustrates: here each embryo apparently was quite normal and possessed its own scutellum.

For *Poa pratensis* he describes the following seedlings: (1) two plumules with only one radicle; (2) two plumules in a single coleoptile with two radicles in a single coleorhiza (illustrated); (3) two plumules each with its own coleoptile, radicle and coleorhiza. In *Poa* he suggests that the polyembryony is connected with the massive suspensor and illustrates sections showing bud-like outgrowths on this as well as from the nucellus.

He also states that in a seedling of corn (i.e. *Zea*) he observed a germinating caryopsis with 'two normal embryos, each with its coleoptile, *scutellum*, coleorhiza and root' (the italics are not in the original).

By far the most significant reference to twin grass embryos is that of Randolph (1936) who, in discussing the development of the maize embryo, states: 'Another type of anomalous kernel development is represented by paired or twin embryos arranged side by side in an otherwise normal kernel. Such grains are found not infrequently in different stocks of maize, and the normal frequency of occurrence



of this type of twinning is markedly increased by X-ray treatment. Since the pericarp and endosperm of these grains show no evidence of doubleness, the twin embryos presumably arise from a single embryo sac. Twin embryo sacs would be expected to produce twin endosperms as well as twin embryos. Furthermore, the twin plants produced by these kernels ordinarily are genetically identical, even in extremely heterozygous stocks, which indicates that they arise from a single zygote, presumably by a division of the entire embryo or of that portion of it from which the plant develops, at some relatively early stage in ontogeny. Seedlings with two plumules and a single primary seminal root undoubtedly owe their origin to an incomplete separation of the embryo into two parts, a separation which involves only the portion which forms the plumule meristem.'

Four other fairly recent papers dealing with twinning in grass seedlings were traced, but all, however, are concerned with the occurrence of haploid, triploid and tetraploid plants amongst such pairs, and in none are there any observations on the anatomy of the caryopses from which the seedlings have arisen, so that the origin of the two embryos must remain obscure.

Ramih, Parthasarathi & Ramanujam (1933) found a large number of twin seedlings in *Oryza sativa*. In one pure line the twin seedlings were as high as 0.1 %. One plant was found to be a haploid, all the rest being normal diploids.

Namikawa & Kawakami (1934) found that twin seedlings occurred at the rate of about 0.05 % in common wheat. Of the twin plants, about a third had one of the twins with abnormal chromosome numbers.

Müntzing (1937, 1938) reported twin seedlings from fourteen species of Gramineae as well as from *Trifolium repens* and *Solanum tuberosum*. Out of 2189 twin seedlings examined, ten had abnormal chromosome numbers in one of the twins. In two sets of *Lolium perenne* twins, both the embryos had the  $3n$  chromosome number. In *Phleum pratense*, he mentions two sets of triplets, all the embryos of which had  $2n$  chromosome numbers.

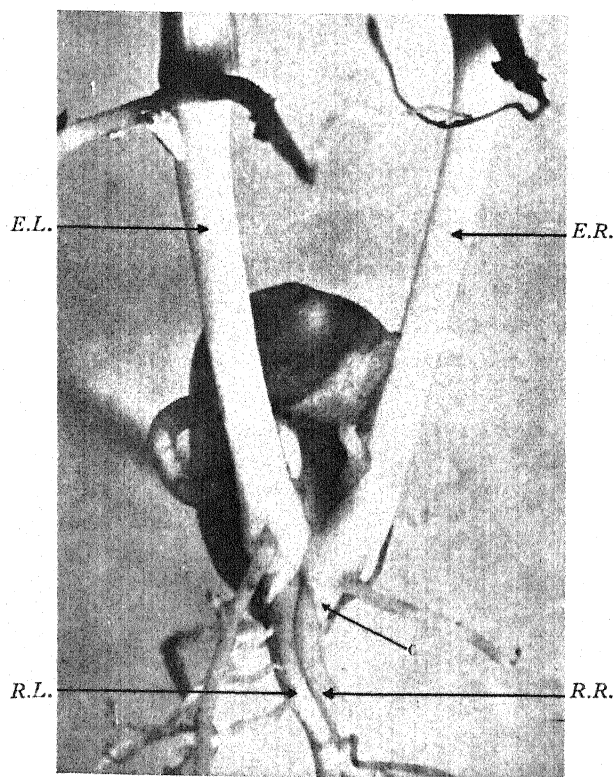
Unfortunately, these papers were not seen until after the maize twin had been fixed, which was done in a vacuome fixative: Attempts were made to observe the chromosomes in the root tips, but with no success. However, the shoot apices were sectioned, and here the nuclei, the cells and the whole apices were similar to each other in size, and were of the same dimensions as those observed in normal shoots. These observations, together with the obvious equality of the two embryos, would suggest that both were identical and were normal plants with the normal  $2n$  number of chromosomes.

#### SUMMARY

The anatomy of a twin seedling of *Zea Mays* is described. Both embryos probably had the normal chromosome complement and appear to have arisen as the result of a constriction or partial longitudinal division of the early pro-embryo stage, giving two complete embryos attached to a single, partially divided scutellum and enclosed in a single caryopsis with a common mass of endosperm.



A



B

A. The twin maize seedlings. B. Part of A enlarged, showing the single caryopsis with the two emerging embryos. *E.L.*, *R.L.* epicotyl and radicle of left embryo; *E.R.*, *R.R.* epicotyl and radicle of right embryo; C, split single coleorhiza.

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## A SIX-STAMENED FLOWER IN *ZEA MAYS* L.

By B. C. SHARMAN

*Department of Botany, University of Leeds*

(With 2 figures in the text)

During the examination of the tassel of a maize plant, a number of flowers were found with more than the usual three stamens. They fell into the following classes:

- (1) A number with two separate stamens plus two stamens with a joint filament, making four anthers in all.
- (2) A considerable number with four distinct stamens.
- (3) Some with three separate stamens plus two 'double' stamens, each made up of two anthers with a joint filament, making five anthers in all.
- (4) Some with four separate stamens plus two anthers with a joint filament, making six anthers in all.
- (5) A few with five separate stamens.
- (6) A single specimen with six separate stamens.

This last seemed so interesting that it was decided to section it in order to obtain an insight into its morphology. The flower, which completely occupied a normally two-flowered spikelet, was fully open when found and showed two glumes, inside which were two more bracts, presumably lemmas. At right angles to the plane of the glumes and lemmas was a pair of membranous structures, which were followed by the six stamens, all six of which arose at the same level and obviously came off the same common tissue.

The sections illustrated in Fig. 1 *a-d* show the relationship of the parts at different levels. Each figure is based on two or three sections in order to compress the stages into as few figures as possible. Fig. 1 *a* is taken just above the separation of the glumes and shows the next two bracts, marked *Y'* and *Y''*, beginning to separate from the rachis: the one on the left will have three bundles, while that on the right is provided with four. In the periphery of the rachis will be seen four separate strands: a pair of which lead to each of the structures placed at right angles to the plane of the glumes and lemmas, and marked *X'* and *X''* in Fig. 1 *c, d*. The remaining vascular tissue, shown shaded in Fig. 1 *a*, is composed of two masses which higher up provide the strands for the six stamens and four lodicules, and even at the level of Fig. 1 *a* are beginning to be organized into separate patches.

Fig. 1 *c* shows the way in which the six stamens are all inserted on the axis in a ring at the same level. A small piece of tissue, marked *Z*, appears in two sections (total height, 12 $\mu$ ). Other than this there is no suggestion of a continuation of the rachis; nor are there any central bundles which might run to any supposedly 'suppressed' parts. Fig. 1 *d* shows the appearance above the separation of the structures *X'* and *X''*, the four lodicules and the six stamens. The bases of the filaments of two sets of two stamens will be seen to be still slightly fused at this level,

but immediately above this they are completely free. Fig. 2 *a*, for comparison, illustrates the relationship of the parts of the two flowers in a normal spikelet.

Although the abnormal flower, as its internal structure shows, is definitely a single structure, it can perhaps best be considered morphologically as being the result of the coalescence of two flowers 'fused back to back'. Normally the spikelets are two-flowered (Fig. 2 *a*), the spikelet axis ceasing growth early after the production of the second lateral floret<sup>1</sup> bud and usually not being traceable above this level in the adult spikelet. This condition is illustrated diagrammatically in Fig. 2 *d*.

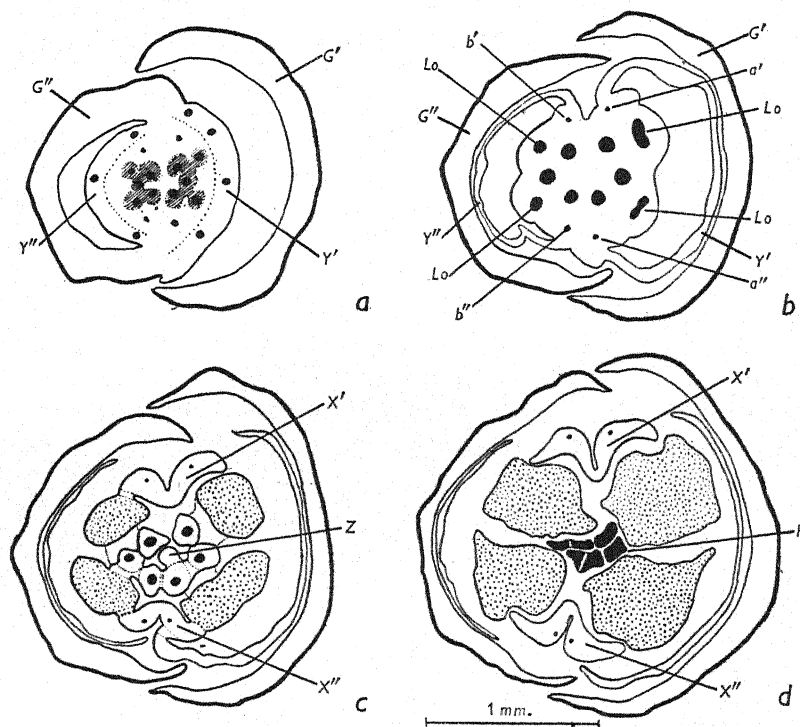


Fig. 1. Transverse sections through the six-stamened male flower of *Zea*. *G'*, *G''*, glumes 1 and 2; *Y'*, *Y''*, lemmas 1 and 2; *X'*, *X''*, palea-like structures; *a'*, *b'* and *a''*, *b''*, vascular bundles for *X'* and *X''*; *Lo*, bundles for lodicules; *Z*, small mass of tissue only 12  $\mu$  high which perhaps may be regarded as being the continuation of the rachis; *F*, filaments of the six stamens.

Occasionally the rachis apex continues actively longer than usual and three lemmas are produced, each having an axillary bud, giving rise to a three-flowered spikelet in the adult, a condition which is illustrated diagrammatically in Fig. 2 *e*. On the other hand, an early cessation of activity leads to the production of a single, pseudo-terminal flower in the spikelet as figured by Malpighi (Arber, 1934) and represented diagrammatically in Fig. 2 *b*. In the six-stamened flower described above, presumably the growth of the spikelet axis ceased rather earlier than usual, so that when the two axillary floret buds developed in the axils of the lemmas, they were

<sup>1</sup> The term 'floret' is used here and below to cover that which develops in the axil of the lemma, i.e. the flower proper (essential organs and perianth as represented by the lodicules) plus the palea or prophyll of the axis on which the flower is born.

free to occupy a more central position than usual, with the consequence that both grew up together as a single, massive, probably slightly indented, meristem. On this arose the two bracts  $X'$  and  $X''$ , each perhaps being initiated from two points but growing up as a single unit by the rapid lateral spread of the initiation, leading to a complete absence of any doubleness in the appearance of the adult structure. Fig. 2 *c* is a diagrammatic illustration of this spikelet for comparison with the single, normal and three-flowered spikelets shown in Fig. 2 *b*, *d* and *e* respectively.

This curious single-flowered spikelet parallels a similar phenomenon reported by Kempton (1913) and Stratton (1923) for the female inflorescence. In the cob the spikelets are also two-flowered; usually the lower one is aborted and only the

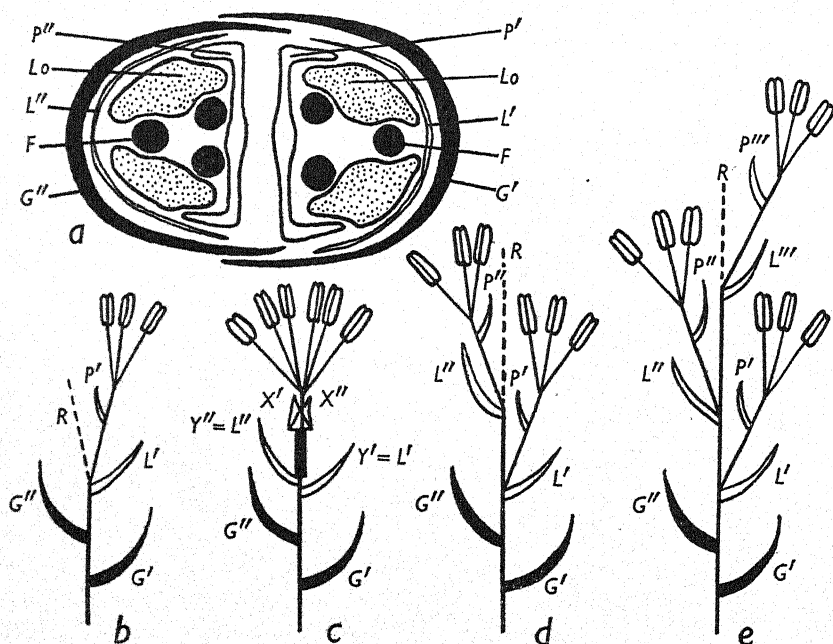


Fig. 2. *a*, Diagrammatic transverse section of normal two-flowered male spikelet. *b*, *d* and *e*, Diagrammatic representations of single-flowered, normal two-flowered and three-flowered spikelets. *c*, Ditto of the flower and spikelet illustrated in Fig. 1.  $G'$ ,  $G''$ , glumes 1 and 2;  $L'$ ,  $L''$ , lemmas 1 and 2;  $P'$ ,  $P''$ , paleas 1 and 2;  $Lo$ , lodicules;  $F$ , filaments;  $R$ , imaginary continuation of rachis;  $X'$ ,  $X''$  and  $Y'$ ,  $Y''$ , parts which are labelled similarly in Fig. 1.

upper one is fertile, though in certain strains (e.g. Country Gentleman) both may develop normally. In a strain grown by the Hopi Indians of Arizona and in a hybrid derived from varieties from China, Salvador and Mexico, Kempton observed what he termed 'connate' grains—single large grains caused by the back-to-back fusion of two embryos and their endosperms, so that a germ appears on each side of the grain. He concludes that 'The development of two ovaries in one spikelet must be simultaneous, as a large number of cases have been found where the two seeds from one spikelet have grown together with a single pericarp', but his illustrations of the mature caryopses suggest that there never has been any actual 'fusion', but rather that the connate grains have arisen from single massive two-ovuled ovaries.

Blaringhem (1920) described as *Zea Mays* var. *polysperma* a strain of maize in which many double grains developed. This variety was examined by Stratton (1923), who showed that the double kernels arose from coalesced flowers, in which both of the flowers of the spikelet were fertile and had developed from the time of their inception in various degrees of 'fusion'. Her figures leave little doubt that this was brought about by the early discontinuance of the spikelet axis, allowing the two laterally developing floret buds to occupy a more central position than usual, and thus grow up as a single two-ovuled unit.

Thus the six-stamened male flower described above is to be regarded as a single flower, probably developed from a single massive apex formed from two lateral buds originating close to each other. Morphologically it can be regarded as being composed of two florets 'coalesced back to back'.

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## THE INTERRELATIONS AND CLASSIFICATION OF THE MYXOPHYCEAE (CYANOPHYCEAE)

By F. E. FRITSCH

*Department of Botany, Queen Mary College, University of London*

(With 5 figures in the text)

The blue-green algae have long been classed in the three orders Chroococcales, Chamaesiphonales, and Hormogoneales. The first of these, especially the series of closely interrelated palmelloid types (*Chroococcus*, *Gloeocapsa*, *Aphanocapsa*, etc.) that constitute the bulk of the order, represents a relatively homogeneous group, but this cannot be said of the other two orders. The appreciable additions to our knowledge of the Algae classed as Chamaesiphonales made during the last twenty years show clearly that they include two distinct series of forms, viz. a group of uni- to multicellular epiphytes for which I propose to retain the name Chamaesiphonales, and a striking series of heterotrichous epiphytes or lithophytes for which the name Pleurocapsales is suggested. In habit these two orders are essentially different. The Chamaesiphonales appear to be closely allied to the Chroococcales and probably represent an offshoot from the same line of evolution. The affinities of the Pleurocapsales are more difficult to determine. The Hormogoneales, distinguished by their filamentous organization and the customary reproduction by means of hormogones, also comprise two sets of forms (Nostocales, Stigonematales) differing in fundamental construction.

### CHAMAESIPHONALES

These, in their simplest representatives, differ from the Chroococcales chiefly in the polarity of the individual cells. The common marine epiphyte *Dermocarpa* (Bornet & Thuret, 1880, p. 74) probably includes among its many species some of the most primitive members of the order. In *D. prasina* and others the protoplast of the mature individual divides successively and along three planes into numerous small bodies (Fig. 1 A, e) which, after acquiring membranes of their own, are liberated as so-called endospores (Geitler, 1925 b, p. 203), in the past often referred to as gonidia. The endospores are comparable to the aplanospores of other Algae and, so far as present evidence goes, germinate immediately and are not capable of passing through a resting period. According to Setchell & Gardner (1919, p. 28) endospore formation takes place simultaneously in certain species of *Dermocarpa*.

A similar method of propagation occurs in diverse Chroococcales, although Geitler (1925 a, p. 359) speaks of this as 'nannocyte-formation' and endeavours to draw a distinction between it and the reproduction by endospores found in *Dermocarpa* and other Chamaesiphonales. The only difference that emerges, however, is that 'nannocyte-formation' in Chroococcales is on our present knowledge a rather rare phenomenon and, when it occurs, usually affects numerous cells of

a stratum, whereas endospore formation commonly takes place only in certain cells; differences in reproductive potentiality between adjacent cells are, however, widespread among Algae. The phenomena themselves appear to be altogether identical in the two instances (cf. Fig. 1 G-K). The distinction is quite arbitrary, and in the following all such examples of the formation of 'nannocytes' are classed

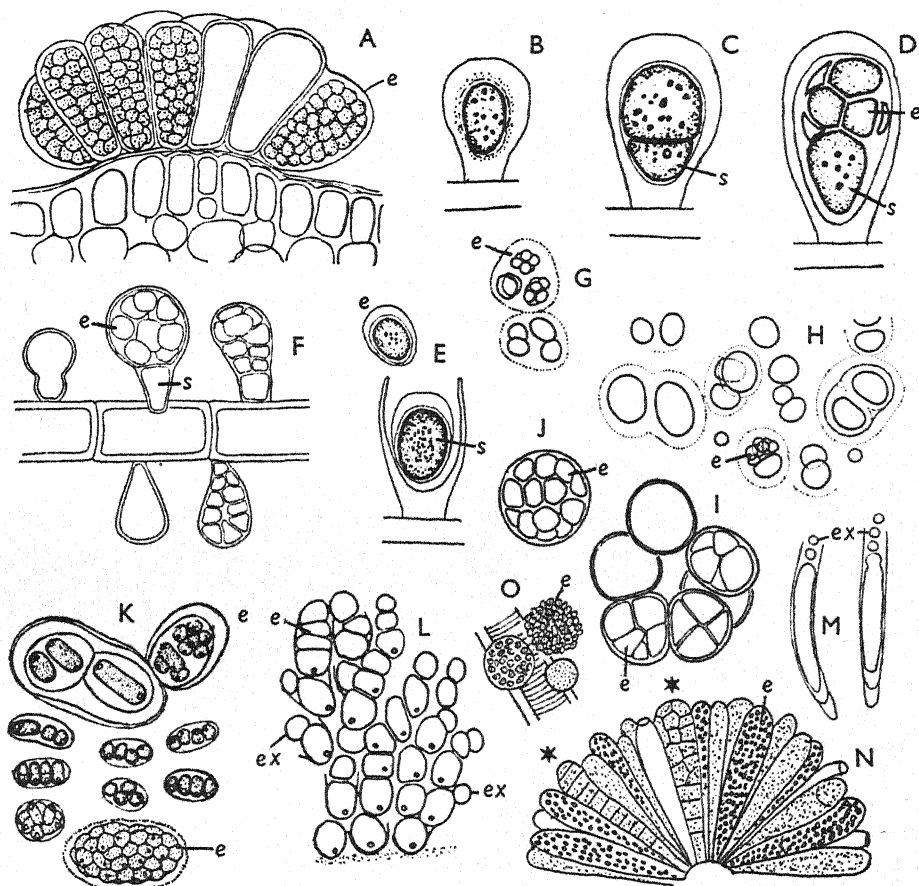


Fig. 1. Chamaesiphonales. A, *Dermocarpa prasina* (Reinsch) Born. & Thur. B-E, *D. incrassata* (Lemm.) Geitl.; B, vegetative individual; C, first division; D, endospore formation in upper portion; E, dehiscence and enlargement of lower sterile portion. F, *D. suffulta* Setch. & Gardn. G, H, *Chroococcus gelatinosus* (Geitl.), vegetative cells and endospore formation. I, J, *Chroococcidiopsis thermalis* Geitl., ditto. K, *Aphanathece caldariorum* P. Richt., vegetative individuals (at top) and endospore formation. L, *Chamaesiphon polymorphus* Geitl., colony showing endospore formation. M, *C. curvatus* Nordst. N, *Dermocarpa protea* Setch. & Gardn. O, *D. sphaerica* Setch. & Gardn. e, endospores; ex, exospores; s, sterile portion of individual. (A after Bornet & Thuret; B-E after Lemmermann; F, N, O after Setchell & Gardner; M after Fritsch; the rest after Geitler.)

as endospores. Among Chroococcales these have been recorded in *Gloeocapsa* (Wille, 1906), *Aphanathece* (Geitler, 1922; cf. Fig. 1 K, e), *Microcystis* (Cannabaeus, 1929, p. 33), and *Chlorogloea* (Geitler, 1925a, p. 359). Moreover, they occur also in *Chroococcus*. Geitler (1935, p. 386) describes from Sumatra a genus *Chroococcidium* which is really nothing else than a *Chroococcus* multiplying by endospores

formed in occasional cells (Fig. 1 G, H, e). It is difficult to find a valid reason for not including this alga in the genus *Chroococcus*, and there is certainly no basis for the distinction of a separate family. A further genus, *Chroococcidiopsis* (Geitler, 1935, p. 391), shows similar endospore formation, but lacks the mucilage envelope of a *Chroococcus* and so far the vegetative division typical of the latter genus has not been observed (Fig. 1 I, J). Whatever be its generic status, it must be regarded as a member of the Chroococcaceae.

Certain *Dermocarpas* have practically spherical cells (e.g. *D. sphaerica* Setch. & Gardn., Fig. 1 O) and these differ from *Chroococcidiopsis* (Fig. 1 I) only in their epiphytic habit and from *Chroococcus* (*Chroococcidium*) *gelatinosus* in the lack of vegetative division and of an offstanding mucilage envelope. These various forms indicate the close affinity between Chroococcales and Chamaesiphonales. The simpler members of the latter may in fact be regarded as chroococcoid forms that have assumed an epiphytic mode of life, resulting in a polar differentiation which finds its expression in basal attachment and apical dehiscence of the fertile individual. Diverse *Dermocarpas*, moreover, agree with *Chroococcus* in exhibiting what is tantamount to a vegetative division. In *Dermocarpa incrassata* (Fig. 1 B-D), for example, the first transverse division of the protoplast results in the delimitation of a sterile lower (s) from an upper portion which alone segments to form endospores (e). After their liberation the remaining half-protoplast enlarges, secretes a new membrane (Fig. 1 E, s), and the same sequence of events is repeated. *D. Leibleinii* (Reinsch.) Born. & Thur. sometimes behaves like *D. prasina* and sometimes like *D. incrassata*, while in *D. suffulta* (Gardner, 1918, p. 440) the lower portion is cut off as a permanently sterile stalk (Fig. 1 F, s).

The characteristic method of spore formation in *Chamaesiphon* is probably to be related to that shown in the species of *Dermocarpa* just considered (cf. also Geitler, 1925b, p. 205). After reaching a certain size the membrane (probably the cell sheath, Fritsch, 1905, p. 198) of the *Chamaesiphon* cell ruptures apically and the exposed protoplast, probably still enclosed in the inner investment, abstricts a series of spherical exospores (Geitler, 1925b, p. 209) successively from its tip (Fig. 1 M), much as conidia are produced in many Fungi. The greater part of the ruptured membrane remains as a cup-shaped or cylindrical envelope around the protoplast. The lower portion of the individual elongates continuously and, while usually remaining short, may in some species (*C. macer* Geitler, 1925a, p. 331) attain to considerable length. The process of spore formation in *Chamaesiphon* differs essentially only from that seen in *Dermocarpas*, like *D. incrassata*, in the fact that the upper fertile portion here gives rise to but a single spore. It can be interpreted as a variant of the method of endospore formation found in the latter genus. Some species of *Chamaesiphon* occasionally show endospore formation (cf. Fig. 1 L, e).

While therefore *Dermocarpa* and *Chamaesiphon* are readily derivable along divergent lines from types like the simple Chroococcales, certain species of the former appear to indicate yet another line of advance. Where the cells are elongate (as in *Dermocarpa protea* Setch. & Gardn., Gardner, 1918, p. 456) there may be a number of transverse divisions of the protoplast (Fig. 1 N, \*) before segmentation sets in in other planes. The freshwater epiphyte *Clastidium* forms its endo-

spores in a single row by transverse division only (Fig. 2 G); in *C. rivulare* Hansg. they are, as in *Dermocarpa incrassata*, commonly formed only by the upper half of the protoplast (Geitler, 1932, p. 410). During the formation of endospores the cell continues to lengthen, and this feature is more marked in the closely related *Stichosiphon* (Geitler, 1932, p. 411; 1935, p. 392; Rao, 1935) where the older individuals may acquire an appreciable length and become transversely segmented into a more or less elongate row of endospores (Fig. 2 A) which are ultimately

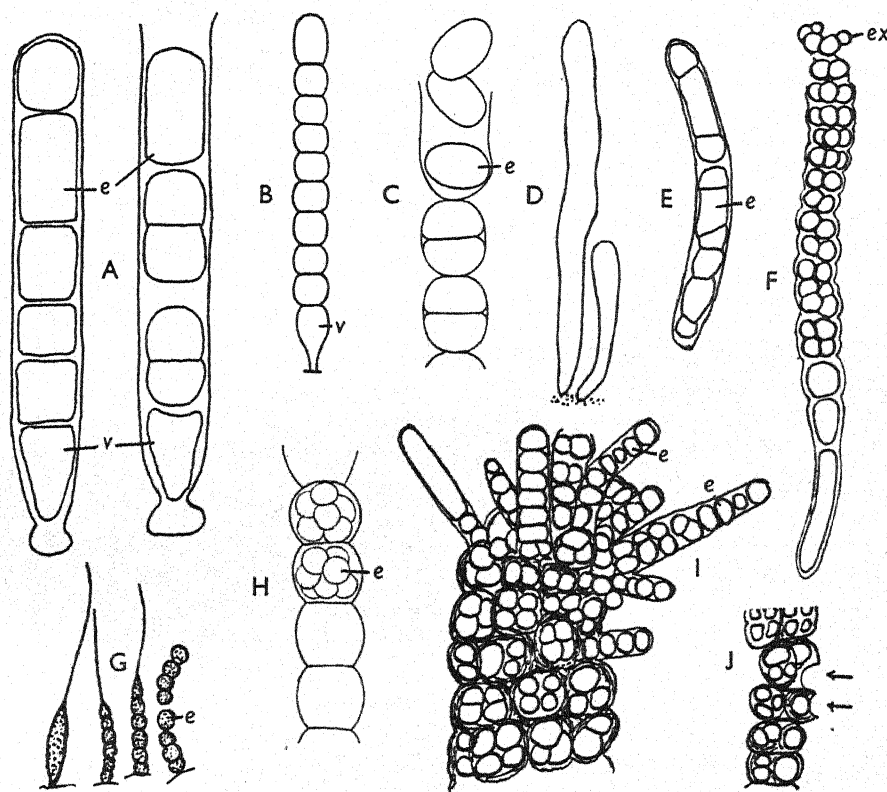


Fig. 2. Chamaesiphonales. A, *Stichosiphon regularis* Geitl., two individuals, that on the right dehiscent. B, C, H, *Endonema moniliforme* Pascher; B, filament; C, cell-division and formation of few endospores; H, formation of many endospores. D-F, I, J, *Siphononema polonicum* Geitl.; D, juvenile stage; E, early stage of endospore formation; F, older individual, formation of cell-packets; I, outgrowth of individual cells of same; J, small part of an older individual showing two places at which exospores have been liberated. G, *Clastidium setigerum* Kirchn. e, endospores; ex, exospore; v, basal cell. (B, C, H after Pascher; G after Kirchner; the rest after Geitler.)

liberated by apical gelatinization of the membrane. The basal part of the protoplast (v) is apparently usually retained. The species of *Stichosiphon* approximate closely to those species of *Chamaesiphon* (cf. *C. polymorphus* Geitler, 1925a, p. 329), which at times produce endospores (Fig. 1 L).

It may well be that the peculiar genus *Endonema* (Pascher, 1929; cf. Fig. 2 B), as yet only recorded from Bohemia, represents a further development of the habit seen in *Stichosiphon*, the species of which merit fuller study than they have received.



Although the cells of *Endonema* commonly produce considerable numbers of endospores (Fig. 2 H, e), there are frequently only two (Fig. 2 C, e); it is by retention of such pairs of endospores that cell-division is accomplished and the multicellular filamentous habit is attained (Fig. 2 C, below).

The remarkably polymorphic *Siphononema* (Geitler, 1925 a, p. 322) probably also belongs here. The continuously elongating juvenile individuals (Fig. 2 D) exhibit progressive formation of endospores (Fig. 2 E), which remain in situ and sooner or later divide along three planes to form *Gloeocapsa*-like packets of cells with stratified orange or reddish mucilage-envelopes (Fig. 2 F); these may be set free by apical breakdown of the surrounding membrane of the original individual. Certain cells of the packets may elongate and form endospores like the juvenile stage (Fig. 2 I, e), while others produce exospores (Fig. 2 F, ex) in the same way as in a *Chamaesiphon*. This striking form appears as an elaboration of the condition realized in *Stichosiphon*, but it is complicated by the production of *Gloeocapsa*-stages; the occasional formation of exospores indicates the close relation between them and the endospores.

#### PLEUROCAPSALES

The members of this order, which have hitherto been classed with Chamaesiphonales on so inadequate a basis as the joint possession of endospores, have no other morphological features in common with that order. Geitler (1932, p. 94) suggests that the Entophysalidaceae (*Entophysalis*, *Placoma*, etc.) among Chroococcales, which afford indications of a filamentous tendency, may illustrate the mode of origin of the Pleurocapsales. The typical members of the latter are, however, very clearly heterotrichous in habit, with a juvenile stage represented solely by a prostrate filamentous growth. There is no evidence of such a habit among Entophysalidaceae, and the mode of derivation of the Pleurocapsales, possibly from Chroococcales, like that of other heterotrichous forms at present remains obscure.

In the more typical Pleurocapsales the heterotrichous habit results in the formation of crusts (*Radaisia*, Sauvageau, 1895; Fig. 3 O) or hemispherical cushions (*Oncobyrsa*, Geitler, 1925 a, p. 350; Fritsch, 1929, p. 185; Fig. 3 M) of microscopic dimensions or of extensive growths visible to the naked eye (*Pleurocapsa*); all the genera mentioned are frequent marine or freshwater epiphytes or lithophytes. The only species of *Pleurocapsa* that has been adequately studied is *P. minor* (Geitler, 1925 a, p. 343). At first the individual consists of a simple thread (Fig. 3 A) creeping over the substratum, but branching results in the formation of a pseudo-parenchymatous prostrate system (Fig. 3 B, C) and development may remain permanently arrested at this stage. As a general rule, however, densely aggregated, and occasionally branched, erect threads arise from this basal system (Fig. 3 D) so that a crust is formed. Growth seems to be apical throughout. The cells of the erect system may segment along three planes or divide obliquely, whereby the filamentous construction becomes obscured. Several of the species of *Pleurocapsa* listed by Geitler (1932, p. 346) would seem to have essentially the same structure (e.g. *P. fluviatilis*, *P. aurantiaca*, *P. minuta*), but others are not so evidently filamentous and it is possible that some of these are really members of Chroococcales.

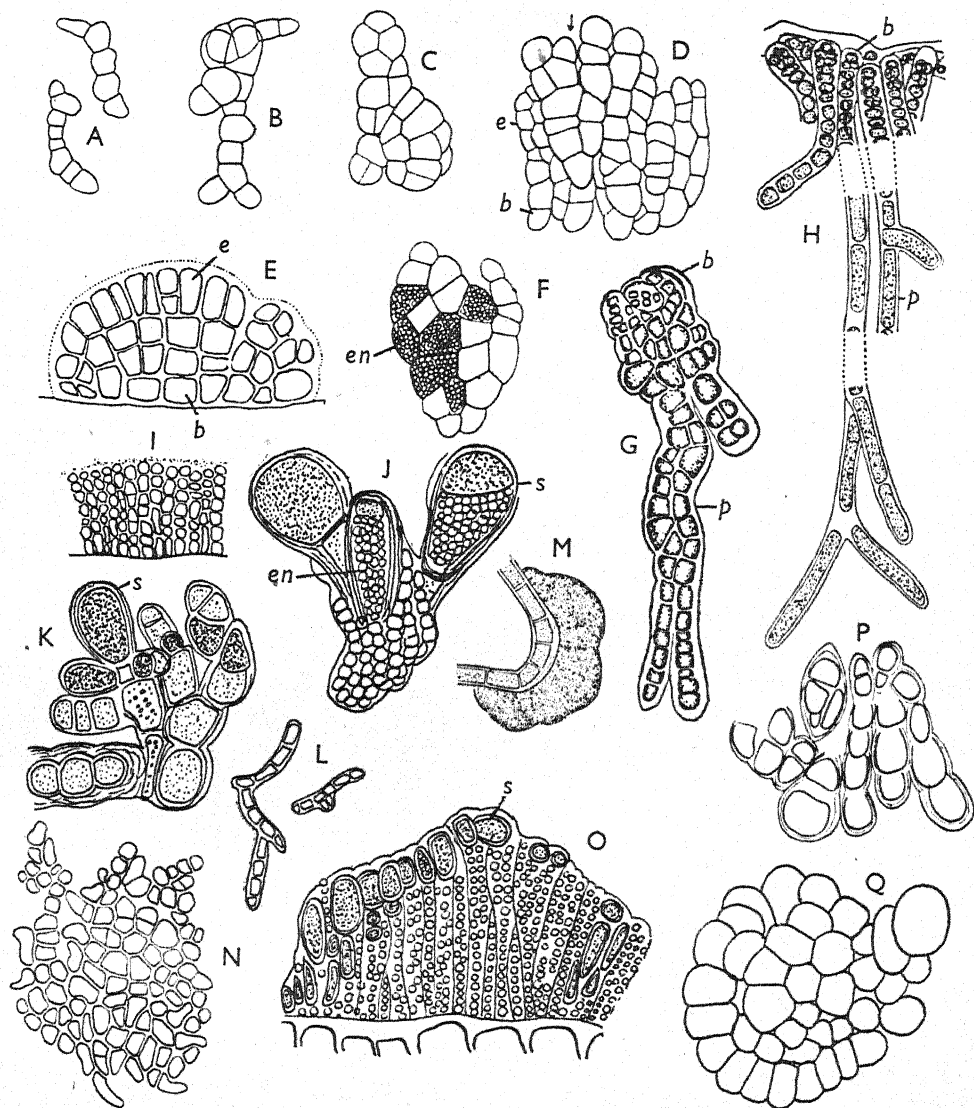


Fig. 3. Pleurocapsales. A-D, F, *Pleurocapsa minor* (Hansg.) Geitl.; A-C, stages in development of prostrate system; D, vertical section of older crust showing branching of erect threads; F, a few of the latter with endospores. E, *Xenococcus Kernerii* Hansg., vertical section of a small crust. G, *Scopulonema Hansgirgianum* Erceg. H, J-L, *Hyella caespitosa* Born. & Flah.; H, superficial (*b*) and penetrating threads (*p*); J, K, part of surface system with sporangia (*s*); L, germinating spores. I, M, N, *Oncobyrsa rivularis* Kütz. emend. Geitl.; I, vertical section of cushion; M, ditto at a lower magnification; N, basal stratum. O, *Radaisia Gomontiana* Sauv. P, *Xenococcus chroococcoides* Fritsch, section of a stratum. Q, *Chroococcopsis gigantea* Geitl. *b*, prostrate and *e*, erect systems; *en*, endospores; *p*, penetrating threads; *s*, sporangia. (G after Ercegović; H, J-L after Bornet; O after Sauvageau; P after Fritsch; the rest after Geitler.)

*Oncobyrsa* (Fig. 3 I, N) and *Radaisia* (Fig. 3 O) show a similar structure to that described for *Pleurocapsa*, but the filaments of the erect system are often more clearly defined. Endospore formation has only been observed in *Pleurocapsa* (Fig. 3 F, *en*) and *Radaisia* (Fig. 3 O), in the former in terminal or more rarely intercalary cells, in the latter within much enlarged terminal sporangia (*s*).

In other epiphytic Pleurocapsaceae the filamentous character is frequently far less well defined. Thus, in *Xenococcus* (Bornet & Thuret, 1880, p. 75; Geitler, 1925*a*, p. 348), with both fresh-water and marine species, the plants are often represented solely by a pseudoparenchymatous basal stratum without any obvious filamentous structure, although its cells may bear short upright forked threads compacted to form a crust (Fig. 3 E, P), giving a habit much like that of an *Oncobyrsa*. In certain species the cells of the basal stratum may be only loosely associated so that the alga presents a colonial, rather than a filamentous, aspect. The tendency to depart from a filamentous construction is paralleled in other heterotrichous series (cf. *Pleurastrum* among Chaetophorales). It is more marked in *Chroococcopsis* (Geitler, 1925*a*, p. 342) in which the cells are aggregated in compact groups (Fig. 3 Q) and only very occasionally form short rows. This is probably a specialized type, never producing an erect system, and parallel to such a form as *Pleurococcus* among Chaetophorales.

All Pleurocapsaceae that have so far become known, therefore, exhibit that development of heterotrichy that results in the formation of crusts, and among these there is a marked tendency towards scanty production or elimination of the erect system. The type exemplified by the heterotrichous filament seems to be lacking. On the other hand, there is an interesting series of endolithic forms which are parallel to the Gomontieae among Chaetophorales. The best known of these is *Hyella* (Bornet & Flahault, 1888; 1889, p. clxv; Huber & Jadin, 1892; Chodat, 1898, p. 446), the species of which are widespread members of the perforating community inhabiting diverse calcareous substrata. The thallus (Fig. 3 H) is differentiated into a first-formed prostrate system (*b*), spreading over the substratum or living within the peripheral layers of the latter, and a system of perforating rhizoid-like filaments (*p*). Sporangia (Fig. 3 J, K, *s*), often of large size, develop from intercalary or terminal cells of the prostrate system. Ercegović (1929, 1930) has described, from dolomitic or other calcareous rocks of the Dalmatian coast, a number of other endolithic genera which present many points of resemblance to *Hyella* and are evidently widespread in the Mediterranean (Berner, 1931, p. 55; Frémy, 1934); according to some opinions (Nadson, 1932, p. 843) they are but forms of *H. caespitosa*. In *Scopulonema* the two systems of threads are much alike (Fig. 3 G), but the cells of the epilithic ones (*b*) soon divide in all directions. The basal system of *Pleurocapsa minor* occasionally produces on its under side short filaments which penetrate into calcareous substrata, and this perhaps gives the key to the origin of forms like *Hyella*.

#### THE HORMOGONEAE

The heterogeneous assemblage of filamentous types included in the Hormogoneae of Thuret (1875) naturally falls into two distinct morphological series, the Nostocales and Stigonematales of Geitler (1925*b*; cf. also Elenkin, 1916). The

distinction, primarily founded on the method of branching, is, on the basis of comparison with other classes of Algae, seen to be more profound and therefore, although Geitler (1932, p. 105) subsequently abandoned it, I am of the opinion that it should be retained.

#### NOSTOCALES

The only characteristic common to the diverse families of this order is the multiplication by hormogonia, although all are filamentous forms of a relatively simple morphological type. The unit of construction is the trichome, a row of undifferentiated and, except in many Nostocaceae, commonly flat cells, separated from one another by septa which are composed only of the often thin inner investment of the cell; the latter is firmly connected with the underlying cytoplasm (Cholnoky, 1937, p. 257). If, as is commonly believed (Fritsch, 1905, p. 196; Mühldorf, 1935, p. 170; 1937, p. 226; 1938, p. 17), the inner investment is but a slightly modified plasmatic membrane, there may be essential protoplasmic continuity between cell and cell. In this sense the thread of an *Oscillatoria* or other similar member of Nostocales can be interpreted as an imperfectly septate derivation of a unicellular individual (Crow, 1922, p. 85; 1928). The continuity of the trichomes is interrupted by the death of occasional cells (concave cells, separation disks), as well as by the production of heterocysts which are found in most Hormogoneae, apart from the Oscillatoriaceae. The mucilage investments which are probably always present, though varying greatly in consistency and commonly taking the form of a firm sheath (Fig. 4 A, B), are no doubt the direct homologues of the envelopes of the Chroococcales. A direct derivation of the diverse Nostocales from unicellular forms of this type may be assumed.

The Oscillatoriaceae and Nostocaceae are characterized by their unbranched filaments with diffuse growth, the latter also by the presence of heterocysts and the frequent production of akinetes. The Rivulariaceae and Scytonemataceae, on the other hand, are distinguished by their false branching (Fig. 4 A, B), the former also by the tapering of the trichomes which often terminate in a hair (Fig. 4 D) and the probable frequent occurrence of trichothallic growth by contrast to the essentially apical growth of Scytonemataceae. The latter family is probably closely related to the type of Oscillatoriaceae represented by the *Lyngbyas* which occasionally show false branching (Geitler, 1932, p. 680; 1935, p. 472), a feature also met with in the Oscillatoriaceous genera *Symploca* and *Porphyrosiphon*. This tendency towards false branching among the non-heterocystous Oscillatoriaceae culminates in *Plectonema* (Fig. 4 A), which is to all intents and purposes a *Scytonema* devoid of heterocysts.

The false branching of Myxophyceae is a process of rejuvenation at certain places within the trichomes and shows many points of parallel with the formation of hormogonia. Both processes are initiated by the dying away of one or more intercalary cells leading to a severance of the trichomes (Bharadwaja, 1933). Hormogonia among Scytonemataceae are mostly formed at the ends of the filaments and, being free at either extremity, their liberation from the enveloping sheath is possible. In the case of false branches, however, only one end of the segment, namely that which grows out into the branch, is free. False branching may thus be regarded as a germination of intercalary hormogonia in situ.



The interrelationships of the Nostocales present a considerable problem owing to the widespread occurrence of heterocysts which, so far as present knowledge goes, possess a specialized and stereotyped structure so that all forms possessed of them must be assumed to have arisen from a common ancestry. It is noteworthy that, in all the heterocystous families, species or genera lacking heterocysts are known. This is so in the rather dubious genera *Isocystis* and *Pseudanabaena* among Nostocaceae, in *Hammatoidea*, *Homoeothrix* (Fig. 4 D), *Amphithrix*, and *Leptochaete* among Rivulariaceae, and in *Plectonema* (Fig. 4 A) and some species of *Spelaopogon* among Scytonemataceae. It would therefore be plausible to regard the heterocystous condition as primary and the non-heterocystous one as secondary. This is the point of view taken by Geitler (1925<sup>b</sup>, p. 219; 1932, p. 94) who regards the Stigonematales as the most primitive and the Oscillatoriaceae as the most advanced among the Hormogoneae of Thuret.

The relatively high degree of morphological elaboration attained by Stigonematales, which led Borzi (1916) to regard them as the most specialized members of Myxophyceae, as well as their heterotrichy (cf. below), scarcely lend support to the view that they stand at the base of the Hormogoneae. The heterocyst is probably a very ancient structure which has a long history behind it. The origin of Nostocales can equally well be sought in simple coccoid types giving rise to an extinct series of multicellular forms, one branch of which diverged prior to the evolution of the heterocyst and led to the Oscillatoriaceae, while another, after the appearance of the heterocyst, gave rise to the other three families of Nostocales and possibly also to the heterotrichous Stigonematales. In other words, the Nostocales can be postulated to have arisen successively from a common group of multicellular forms which were possibly something like the present-day *Oscillatoria* and have persisted as the Oscillatoriaceae. The Scytonemataceae and Nostocaceae, which seem to be reasonably homogeneous, probably represent divergent evolutionary series. The Rivulariaceae, on the other hand, appear to include genera, which are possibly more closely related to members of Scytonemataceae (*Calothrix*, *Dichothrix*) or Nostocaceae (*Gloeotrichia*, *Rivularia*) than to one another.

The fossil types referred to blue-green algae, if they actually belong to this class, as well they may, afford no morphological data that help in the elucidation of the evolutionary sequence. None of them, as far as I am aware, show either of the characteristics (presence of a firm sheath and of heterocysts) that would alone render a definite reference possible, and none of the filamentous types belong recognizably to the Stigonematales.

#### STIGONEMATALES

The members of this order are distinguished from other Hormogoneae not only by the abundant occurrence of true branching, albeit occasionally accompanied by some false branching, but also by the marked heterotrichy exhibited by many of them. In this respect there is often appreciable resemblance to some Pleurocapsales but, apart from other differences, the Stigonematales nearly always possess heterocysts and in many of them at least there are definite pit connexions between the cells. The threads for the most part exhibit apical growth. It is noteworthy that the Stigonematales are practically absent from the sea and that

they exhibit their greatest development in warmer climates. Many species are terrestrial.

The heterotrichous habit is plainly recognizable in several, unfortunately rare and little known, genera (Pulvinulariaceae) which appear to represent the more primitive forms. *Pulvinularia* (Borzi, 1916, p. 574), an epiphyte on *Fontinalis* as

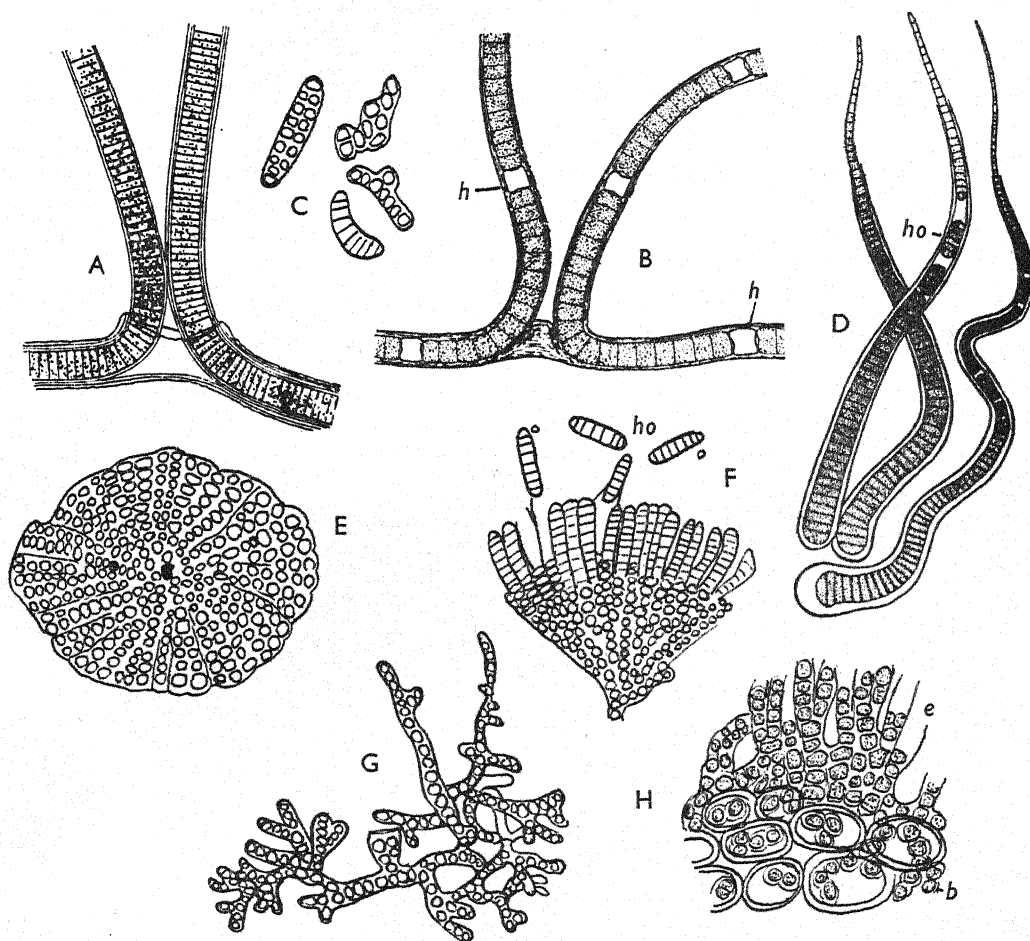


Fig. 4. A, *Plectonema Tomasinianum* Born., false branching. B, *Scytonema ocellatum* Lyngb., ditto. C, E, F, *Pulvinularia suecica* Borzi; C, young stages; E, prostrate system; F, older plant in profile, showing hormogone formation. D, *Homoeothrix juliana* (Menegh.) Kirchn. G, *Hyphomorpha Antillarum* Borzi. H, *Capsosira Brebissonii* Kütz., basal part of a plant with endophytic prostrate system. b, prostrate and e, erect system; h, heterocyst; ho, hormogonia. (A after Bornet; B and D after Frémy; the rest after Borzi.)

yet only recorded from Sweden, has the same habit as *Oncobyrsa*. The first-formed basal system, originating from the germinating hormogones (Fig. 4 C), is a one-layered disk composed of fused threads radiating from a central point (Fig. 4 E). From it there later arise numerous erect threads (Fig. 4 F) which are laterally conrescent, the whole forming a small rounded cushion. In *Hyphomorpha* (Borzi,

1916, p. 582), an epiphyte on a tropical *Trichocolea*, there is only the prostrate system (Fig. 4 G); the older threads, as in so many Myxophyceae, tend to become resolved into chroococcoid groups.<sup>1</sup> A third genus, *Loriella* (Borzi, 1892, p. 44; 1916, p. 570), so far only observed on human skulls from Papua, is stated to be entirely erect-growing, but the existence of an evanescent basal system is not out of the question. The three genera are distinguished by the forked branching of the erect threads when these are present.

Well-marked heterotrichy is also seen among Capsosiraceae in *Stauromatonema* (Frémy, 1929, p. 385; Geitler, 1935, p. 411), the species of which form flat crusts

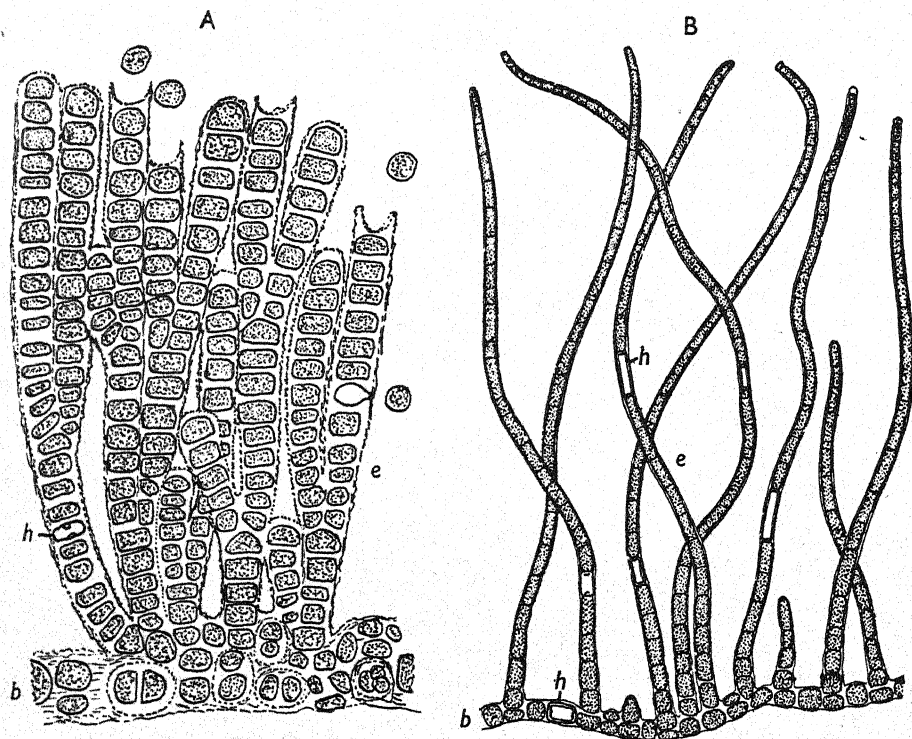


Fig. 5. A, *Stauromatonema nigrum* Frémy. B, *Hapalosiphon hibernicus* W. & G. S. West. b, prostrate and e, erect systems; h, heterocyst. (After Frémy.)

on rocks in tropical fresh waters. The first-formed part of the thallus is a pseudo-parenchymatous stratum composed of contorted threads, while the numerous closely approximated erect threads show occasional lateral branching (Fig. 5 A). One species lacks heterocysts. In *Capsosira* (Borzi, 1917, p. 23; Frémy, 1930, p. 383) the basal system (Fig. 4 H, b) usually ramifies among the substance of the substratum (other water plants, dead wood), while the numerous erect threads are combined to form minute, somewhat gelatinous, cushions.

The Nostochopsidaceae, a family of specialized forms distinguished by the frequent position of the heterocysts at the ends of short laterals, show no indica-

<sup>1</sup> *H. Perrieri* Frémy (1927) cannot be included in this genus, as defined by Borzi.

tions of heterotrichy, but this is again seen in many of the less advanced Stigonemataceae (Fig. 5 B). In these the erect threads (e), as a result of more active growth, frequently differ from the prostrate ones (b) in their narrower and longer cells. The erect threads are, moreover, commonly little branched and it is they alone that produce hormogonia, while the akinetes found in several genera are formed by cells of the prostrate system. The two parts of the heterotrichous filament are thus more clearly differentiated in these Stigonemataceae than they usually are, and this indicates a relatively high degree of specialization. Heterotrichy of the type just described is well shown by *Westiella* (Borzi, 1917, p. 84; Frémy, 1929, p. 434), *Hapalosiphon* (Fig. 5 B), and *Fischerella* (Bornet & Thuret, 1880, p. 155; Gomont, 1895, 1902; Frémy, 1929, p. 439). The first of these is uniseriate throughout, while *Hapalosiphon* shows only occasional horizontal division of the cells of the prostrate system; in *Fischerella* the rounded cells of the latter are commonly arranged in several rows, a condition which is characteristic of most *Stigonemas*.

The type of heterotrichy exhibited by such Stigonematales as *Pulvinularia* (Fig. 4 E, F) and *Stauromatonema* (Fig. 5 A) recalls that seen in the Pleurocapsales; the differences lie in the presence of pit connexions, in the usual presence of heterocysts, and of hormogonia in *Pulvinularia*. The two genera mentioned, moreover, occupy the same general habitats as the Pleurocapsales. In view of our inadequate knowledge of these genera, and especially of the Pleurocapsales, one hesitates to accept the implications suggested by these resemblances, but it does not appear to be altogether out of the question that the Pleurocapsales may be descendants from the simple Stigonematales adapted to a special habitat. It is especially *Pulvinularia* whose further study would be of interest from this point of view.

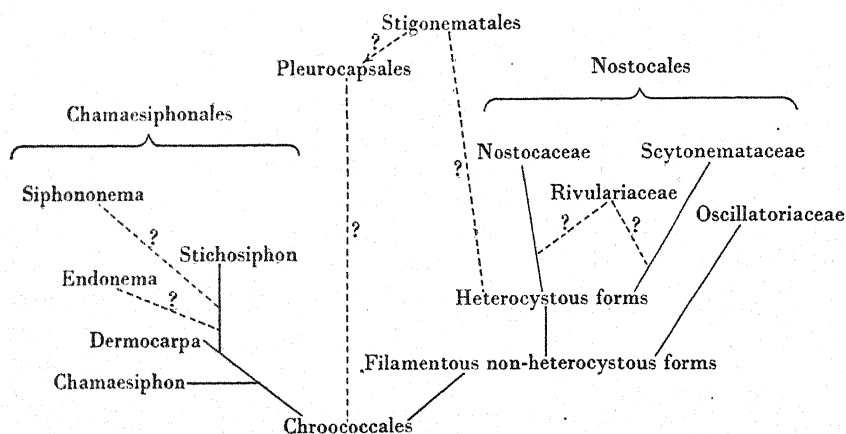
It is equally possible, however, that Pleurocapsales and Stigonematales are but parallel developments from diverse origins. Geitler is of the opinion that the genus *Siphononema* (p. 138), which in certain stages shows resemblances to the older parts of a *Stigonema* with their gloeocapsoid groupings, may illustrate a mode of origin of Stigonemataceae from forms comparable to the Chamaesiphonales and that the simpler, more definitely filamentous, members of Stigonematales are derived. The resemblances between *Siphononema* and *Stigonema* appear, however, to be altogether superficial, and it does not seem credible that so advanced a morphological form as *S. mammosum*, for instance, could have evolved from anything like a *Siphononema*.

The relation of Stigonematales to Nostocales is difficult to assess and involves the whole problem of the relation between heterotrichous and non-heterotrichous filamentous forms which is discussed elsewhere (Fritsch, 1942). The derived condition of the non-heterotrichous types, which is possible in certain classes, appears less probable in Myxophyceae, because there is no evidence that these (i.e. Nostocales) show a greater specialization in any respect than the heterotrichous Stigonematales. It is to be noted that certain genera of Scytonemataceae (*Spelaeopogon*, *Seguenzaea* Borzi, 1917), with prevalent false branching, show a heterotrichous filamentous habit, and such forms suggest a possible derivation of Stigonematales from the same ancestry as gave rise to the Nostocales.



## SUMMARY

The blue-green algae hitherto classed as Chamaesiphonales include two distinct series of forms which probably have only a remote affinity with one another. One series, for which the name Chamaesiphonales is retained, includes a number of characteristic epiphytes (*Dermocarpa*, *Chamaesiphon*, *Stichosiphon*), some of which approximate closely to the Chroococcales. It is concluded that what Geitler calls 'nannocytes' among Chroococcales are in every way equivalent to endospores. The Chamaesiphonales possibly led on to such relatively complex types as *Endonema* and *Siphononema*. The other series, the Pleurocapsales, comprises diverse filamentous forms of a heterotrichous habit (*Pleurocapsa*, *Oncobyrsa*, etc.), as well as reduced types (*Xenococcus*, *Chroococcopsis*) and lime-boring forms (*Hyella*, *Scopulonema*). The affinities of the Pleurocapsales are obscure, but the possibilities of a derivation from Stigonematales are discussed.



Scheme of possible interrelationships of Myxophyceae.

The Hormogoneae likewise include two separate morphological series, the Nostocales and Stigonematales, the latter distinguished not only by their true branching, but more clearly by the evident heterotrichy of the majority, and especially of the less specialized, of its members. The relatively high organization of the Stigonematales is not in favour of Geitler's view that they are primitive and that the families of Nostocales are derived from them. It is equally possible that the latter are derived from a primitive series of *Oscillatoria*-like forms persisting to the present day as the Oscillatoriaceae and giving rise, after the appearance of heterocysts, to the Nostocaceae, Scytonemataceae, and Rivulariaceae, and perhaps also to the Stigonematales. The close relation of Oscillatoriaceae and Scytonemataceae is pointed out and the false branching distinctive of the latter is interpreted as a modification of the process of hormogone formation. The Rivulariaceae are regarded as heterogeneous.

The following is an epitome of the primary classification of Myxophyceae advocated:

I. *Chroococcales*: unicellular or colonial (commonly palmelloid); multiplication by cell-division and by endospores.

II. *Chamaesiphonales*: unicellular or colonial epiphytes or lithophytes exhibiting marked polarity; multiplication by endospores or exospores.

III. *Pleurocapsales*: heterotrichous filamentous types devoid of heterocysts; multiplication by endospores.

IV. *Nostocales*: non-heterotrichous filamentous types, often showing false (rarely true) branching; heterocysts commonly present; multiplication by hormogones, hormocysts, and akinetes.

V. *Stigonematales*: heterotrichous filamentous forms showing true branching, mostly with heterocysts and usually showing clear pit connexions between the cells; multiplication by hormogones and hormocysts, more rarely by akinetes.

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## REVIEW

*An Introduction to the Study of Algae.* By V. J. CHAPMAN.  $8\frac{1}{2} \times 5\frac{1}{4}$  in. Pp. 287, with 209 figures in the text. Cambridge University Press, 1941. Price 18s.

Dr Chapman's book, *An Introduction to the Study of Algae*, is a courageous attempt to supply a need long felt by students. It is, doubtless, much easier to criticize a book when it is written than to write one which successfully avoids the pitfalls lying in the path of an author who ventures into the algal field.

Perhaps because the plants forming the subject of the book occupy a relatively lowly position on an evolutionary scale, they present an assemblage of forms differing among themselves in minute but significant detail, living in conditions not fully comprehended, and, in many cases, undergoing life cycles which have not yet been traced. Add to this the problem of correlating literature in a variety of languages, relative to plants in varied habitats, with some uncertainty, at least in the earlier works, as to identity of species discussed, and the difficulties of presenting knowledge of the algae in coherent, logical and readily appreciable form become at once apparent.

In attempting such a task two forms of book may be considered. One is the 'magnum opus' in which every known fact is faithfully recorded and which automatically becomes an algal encyclopædia, giving extremely useful service to advanced workers but providing, by its very nature, somewhat indigestible fare and lacking the 'readable' quality essential to the needs of the non-specialist student of algology. The opposite end of the scale is held by the superficial short book, written on broad lines, of very general interest, making no claim to be anything more than a simple introduction to the subject.

Anything in between these two extremes involves the problem of selection. Herein has lain Dr Chapman's greatest difficulty. No two authors would necessarily make the same selection and many teachers of algology would not accept Dr Chapman's selection. There is always an advantage in escaping from the stereotypic, and this Dr Chapman has succeeded in doing. But in selecting types, a certain just balance between significance and occurrence must be maintained, and in this Dr Chapman is not wholly successful. Some well-known types have been rejected on the grounds that a student is rarely likely to meet them, yet others equally unlikely are treated in great detail—an inconsistency not always justified by the implications of the facts described.

This inequality of treatment is a feature of the book throughout. The author devotes 95 pages to the Chlorophyceae (Charales 5 pages), 85 pages to the Phaeophyceae and just 12 pages to the Xanthophyceae, Bacillariophyceae, Chrysophyceae, Cryptophyceae and Dinophyceae.

Dr Chapman has attempted to forestall criticism of these points by pointing out in his introduction that the content of the book has been selected to meet the needs of students and contains matter for about 24 lectures covering courses planned for students reading for degrees. The author admits that inequality of emphasis has been deliberately accepted in order to keep the book within reasonable bounds for the avowed purpose.

The book is, however, not a course of lectures. It is not written in that form, and its matter, despite the system of asterisks, surely far exceeds what might reasonably be expected of an undergraduate student in an examination. The book comes into the category of a general treatise and, despite the introduction, is open to criticism on that basis.

Dr Chapman also states in his introduction that the author 'firmly believes in this medium [illustration] as the best means of teaching'. Yet his use of illustration belies the claim. The figures, though good in themselves, are most lamentably overcrowded and so much reduced in size that their usefulness as a teaching medium is very seriously impaired. Moreover, there is not adequate reference in the text to the figures, nor are the explanations below the figures in all cases sufficiently descriptive.

The section of the book which deals with systematic description, the hardest task of all, has obviously proved difficult writing for Dr Chapman, and he is less successful here than in other parts of the book. The chapters in question are dull and uneven. The inequality is due to the fact that here and there researches in one limited field have been fully described, while work in another



field with an equal claim on a student's attention has been wholly omitted. In actual presentation, the writing of this section fails to reach the standard one would have expected. The author is not at his ease: his descriptions do not give clear mental pictures, so essential to good teaching, which a student can readily grasp. Facts which are themselves arresting and might have been used to challenge imagination are passed over without comment. Unproven hypothesis is, here and there, made to assume the guise of statement of fact.

In contrast to the earlier part of the book, the later chapters hold the reader's interest more closely. The author escapes from the burden of type description and writes in much happier vein, especially where, as in the field of marine ecology, he is on more solid ground of personal experience. Here he writes with conviction and arouses real interest. At this point, Dr Chapman has done a real service to students and will doubtless succeed in stimulating an attraction towards research in this field. Fresh-water algal ecology is not treated with the same degree of attention however, and there is much in the chapter on evolution to which one would hesitate to subscribe.

On the whole the book bears evidence of a failure to appreciate the added value which careful presentation gives to good matter. The lack of balance is so marked that it challenges the attention of the reader, and, in some cases, the views expressed are distinctly open to question; but there are some good chapters, and there is no question that the book will serve a very useful purpose. Students for whom it is primarily written will find it of great assistance. It will relieve them from the laborious and time-consuming task of compiling information from a large number of original sources.

M. J. KNIGHT

## EXPERIMENTAL TAXONOMY

V. CYTOLOGICAL STUDIES IN SEA PLANTAINS ALLIED  
TO *PLANTAGO MARITIMA* L.

By F. EARNSHAW

*Edinburgh and East of Scotland College of Agriculture*

(With 5 figures in the text)

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## I. INTRODUCTION

The previous contributions to this series of papers have reported the findings of an experimental evolutionary study of populations of sea plantains allied to *Plantago maritima* L. carried out by Dr Gregor and his colleagues (Gregor, Davey & Lang, 1936; Gregor, 1938, 1939; Davey & Lang, 1939).

The investigations have emphasized the significance of the local population as a unit in evolution. Individuality of discrete populations, often perceptible only by the *majority* of individuals of a population resembling each other more than they resemble the *majority* of individuals of another population, is initiated by factors which control the extent of intercommunity breeding. Though these controlling factors are liable to change, the constitution of some populations may be such as permanently to prevent their free interbreeding under any spatial or ecological circumstances. Migration and environmental changes may alter the extent to which genes can be exchanged between communities which are potentially interfertile. It would clearly be impracticable to recognize such dynamic populations in the orthodox taxonomic manner. Hence the need was felt for a system of population classification which could record the status of populations

and the kind and degree of isolation which keeps them apart, biological values of which present taxonomic definition does not take cognizance. The units of experimental taxonomy were therefore defined on the basis of the ability of populations to exchange genes; in other words, the visible attributes of populations were used only in so far as they provided a clue to the manner of population differentiation.

It will be readily appreciated that in order to assess the relative importance of these differentiating processes it is essential to determine the breeding structure of the plantain population as a whole and to ascertain the potentialities for gene exchange between its constituent communities. The objects of the present investigation were to examine the cytological aspects of this problem. Quoting unpublished data of Miss D. McCullagh, Miss E. S. Bennett and the present writer, Gregor (1939) pointed out that whereas North American, Greenlandic and north European sea plantains are apparently exclusively diploid ( $x=6$ ), populations inhabiting the European Alpine region are both diploid and tetraploid ( $x=12$ ). In the same paper it was shown that all diploid populations exhibited a high degree of interfertility, and that different samples of tetraploids could be bred freely together. Thus between the populations within these two groups there is no inherent barrier to gene exchange, although in nature such interchange may never be realized for other reasons.

The position with regard to the exchange of genes between the diploids and tetraploids was more complicated. It had been established that diploids and tetraploids can be crossed, but it was not known whether such crossings could actually initiate an exchange of genes between the two populations. Consequently the diploid and tetraploid groups were provisionally regarded as separate *coenospecies* (i.e. groups unable to exchange genes) with the proviso that 'if it should be found that diploid and tetraploid groups are capable of exchanging genes, they would comprise separate *ecospecies* of the same *coenospecies*'.

## II. MATERIAL AND METHODS

The methods of sampling wild populations have been fully dealt with by Gregor, Davey & Lang (1936), and will not be detailed here. Throughout the text samples of wild origin are referred to under their sample numbers. Specimens of botanic garden origin, kindly sent by the late Miss McCullagh, retain the specific names under which they were received. The provenance of all types is shown in Table 1. Of each of samples 116, 118 and 119 one hundred plants were available; for the remaining numbers six plants were chosen at random from samples examined in previous years, whilst the hybrid progenies were represented by up to twenty plants.

Cuttings rooted in *Sphagnum* or potted seedlings provided root-tip material which was fixed in Langlet's modification of Navaschin's fluid. After dehydration and embedding according to La Cour's (1931) schedule, sections were cut at  $8\mu$  and stained by the gentian violet-iodine technique.

Pollen mother cell material usually smeared with difficulty, and the method could be used only in a small number of favourable cases. Permanent smears were fixed in 2 BE or acetocarmine. Paraffin embedding was finally adopted as the routine method for anthers. Of the various fixatives tried, Belling's (1930) modification II of Navaschin proved to be the most generally satisfactory. Occasionally better results were obtained with S2 Smith (Lee, 1934) or with 2 BE.

Drawings were all made at bench level with a camera lucida using a Watson holoscopic oil-immersion objective and a 20× eyepiece, giving a magnification of 1300 diameters. Except where otherwise stated drawings are from preparations fixed in Belling II.

## III. CYTOLOGICAL DATA

(1) *Somatic chromosomes*. It will be seen from Table 1 that the plantain populations inhabiting the Alpine region are of two kinds: (a) diploid ( $n=6$ ) and (b) tetraploid ( $n=12$ ); and that, with the exception of the Alpine sample P119, which contained approximately 4% of diploids, population samples were either all diploid or all tetraploid.

Table 1. *Chromosome numbers*

Population samples and source	Chromosome no.		Hybrids	Source	Chromosome no.	
	Mitosis	Meiosis			Mitosis	Meiosis
P81 } Atlantic	12	—	C21 ( $F_2$ )	$F_2$ population	24	12
P82 } coast,	12	6	C22	$P. alpina$ (Edin.) × P63	24	12
P84 } U.S.A.	12	6	C23	P63 × $P. alpina$ (Edin.)	24	—
P21 } Britain	12	—	C17	$P. serpentina$ × P21	12	—
P91 }	12	—	C28	$P. carinata$ × PsS9	12	6
P104 } Iceland	12	—	C28 ( $F_2$ )	$F_2$ population	12	—
P63 }	24	12	C17 ( $F_2$ )	$F_2$ population	12	—
P65 }	24	12	C21	$P. alpina$ (Edin.) × PsS9	24	—
P111 }	12	—	C29	PsS9 × P84	12	6
P113 }	12	6	C32	$P. carinata$ × P84	12	—
P116 (a) } Alps	12	—	C37	$P. alpina$ (Edin.) × P81	18	9
P117 (a) }	24	—	C37 (N.S.)	Natural seed off C37	24	—
P117 (a) }	24	—	C38	$P. alpina$ (Edin.) × P84	18	9
P118 (a) }	12	—	C38 (N.S.)	Natural seed off C38	24	12
P118 (a) }	12	—	C39	$P. alpina$ (Edin.) × P91	—	9
P119 }	12 & 24	—	C43	P113 × P104	12	—
* $P. alpina$ (Edin.)	24	12	C46	P113 × P65	24	—
* $P. carinata$	12	6	C46 (N.S.)	Natural seed off C46	24	—
* $P. serpentina$	12	6	C56	PsS9 × P113	12	—
†PsS9	12	6				

\* Botanic Garden specimens received from Miss D. McCullagh.

†  $P. serpentina$  selfed.

The progeny of diploid and tetraploid parents were, as expected, diploid and tetraploid respectively. Hybrids between diploids and tetraploids showed the triploid number ( $n=9$ ) in C37, C38 and C39, but two plants, C21, tetraploid ♀ × diploid ♂, and C46, diploid ♀ × tetraploid ♂, were tetraploid. The progeny of the triploids, C37 N.S. and C38 N.S. also proved to be tetraploid.

The mitotic chromosomes were quite slender with median to subterminal centromeres, and the complements of members of different populations were in all cases similar in appearance. In the diploid individuals a satellite was frequently present on two subterminally constricted chromosomes. Sometimes three such chromosomes were observed in triploids but more than two were not observed in any tetraploid. In all cases, however, the satellite was very small, and its absence from some cells and individuals was probably due to local fixation effects.

(2) *Meiosis in diploids*. Samples P82, P84, P113,  $P. carinata$ ,  $P. serpentina$  and PsS9. Critical observation of stages earlier than diakinesis was precluded by the small size of the chromosomes, but the metaphase behaviour in all types was uniform. The twelve chromosomes were always found as six bivalents, most frequently each with a single terminal chiasma, though a proportion of cells in all plants had one or at most two



bivalents with two terminal chiasmata (Fig. 1*a, d, e*). In Table 2 will be found figures for the chiasma frequencies per cell and per bivalent for sample P82, *P. carinata* and PsS9. Slightly precocious separation of some bivalents did not disturb the numerically regular segregation at first anaphase. A single chromatid bridge was seen in each of a few cells at late anaphase I, and again in a few cells at anaphase II in *P. carinata* (Fig. 1*b, c*). In no case was an acentric fragment found to accompany the first anaphase bridge.

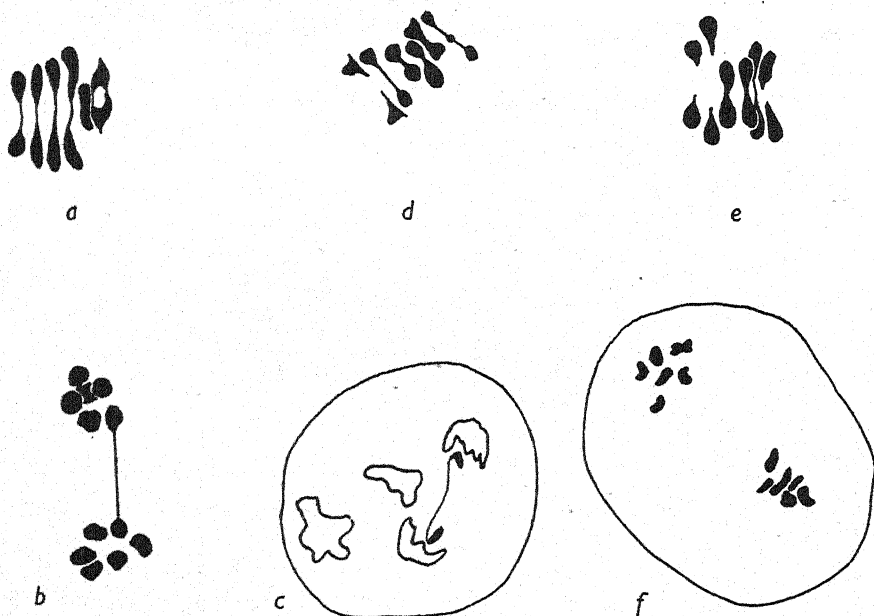


Fig. 1. Diploid hybrids. *P. carinata*: (a) metaphase, (b) anaphase I, (c) anaphase II—(b) and (c) showing bridge chromatids—(d) metaphase; *P. serpentina*: (e) metaphase I, (f) (fixed 2BE) metaphase II.



Fig. 2. Diploid hybrids. C28: (a) metaphase; C29: (b) tetraploid(?) pollen mother cell.

The behaviour at meiosis in the diploid hybrids C28 (PsS9  $\times$  *P. carinata*) and C29 (PsS9  $\times$  P84) differed in no way from that described above, there being no trace of failure of pairing (Fig. 2*a*). In one individual of C29 a single cell at premetaphase was seen with more than twelve chromosomes and, although unfortunately all twenty-four chromosomes could not be counted, it seems certain that this was a tetraploid pollen mother cell resulting from syndiploidy in an archesporial cell (Fig. 2*b*).

(3) *Meiosis in tetraploids*. Samples P65, P63 and *P. alpina* (Edin.), C21 (*P. alpina* (Edin.)  $\times$  PsS9), C22 (P63  $\times$  *P. alpina* (Edin.)). Never more than two quadrivalents per cell were observed in any case, the remainder of the twenty-four chromosomes usually forming bivalents of similar configuration to those in the diploids (Fig. 3a, b, c). The

Table 2. *Chiasma frequencies of diploids and diploid hybrids*

Sample	No. of cells	No. of cells with			No. of bivalents with		Mean no. of Xta per bivalent
		6 Xta	7 Xta	8 Xta	1 Xa	2 Xta	
P82	20	8	12	—	108	12	1.100
<i>P. carinata</i>	128	85	35	8	717	51	1.066
PsS9	23	10	9	4	121	17	1.123
C28	125	50	62	13	662	88	1.117
C29	110	49	55	6	593	67	1.102

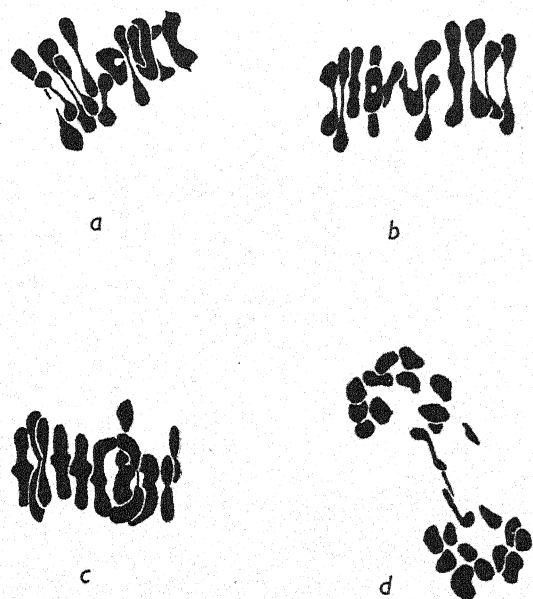


Fig. 3. Tetraploid types. P63: (a) metaphase; *P. alpina* (Edin.): (b) and (c) metaphase, (d) anaphase I—bridge and fragment.

quadrivalents were simple chains or rings with three or four chiasmata respectively. Occasionally a quadrivalent was replaced by a trivalent and univalent. The maximum chiasma formation of sixteen per cell was observed once only in a cell of *P. alpina* (Edin.) which had eight rod bivalents and two ring quadrivalents. Anaphase separation was usually regular as the following distributions show:

	Sample P63	<i>P. alpina</i> (Edin.)
12 : 12	12 cells	9 cells
11 : 13	0 cells	1 cell

Chromatid bridges at anaphase I were found in *P. alpina* (Edin.). Two cells each showed a single bridge with fragment (Fig. 3*d*) and two others each had a broken bridge without a fragment.

Table 3. *Chiasma frequencies of tetraploids and tetraploid hybrids*

Sample	No. of cells	No. of cells with					No. of potential bivalents with		Mean no. of Xta per potential bivalent
		12 Xta	13 Xta	14 Xta	15 Xta	16 Xta	1 Xa	2 Xta	
P 63	26	7	14	4	1	—	287	25	1.080
<i>P. alpina</i> (Edin.)	54	11	18	19	5	1	575	73	1.113
C 21	23	5	10	6	1	1	247	29	1.105
C 22	12	1	6	4	—	1	126	18	1.125



Fig. 4. Tetraploid hybrids. C 21: (a) metaphase I, (b), (c) and (d) anaphase I—(b) two single bridges, (c) single bridge and fragment, (d) double bridge and two (?) fragments; C 22: (e) metaphase I, (f) anaphase I—bridge chromatid.

The meiotic behaviour of non-hybrid tetraploids was paralleled in the tetraploid hybrids C 21 (*P. alpina* (Edin.)  $\times$  PsSg) and C 22 (P 63  $\times$  *P. alpina* (Edin.)) (Fig. 4*a, e*, and Table 3). Following are some exact counts of anaphase segregations:

	C 21	C 22
12 : 12	15 cells	23 cells
11 : 13	2 cells	1 cell
12 : 11 and 1 lagging	3 cells	0 cells

In C21, amongst 200 cells at first anaphase two were seen each with a single bridge and fragment (Fig. 4c), whilst a third (Fig. 4b) had two bridges involving separate bivalents though no fragments were visible. In a fourth cell a double bridge involving a single bivalent was found, but it could not be determined whether the adjacent fragment was single or represented two fragments in close conjunction (Fig. 4d). At anaphase II only one cell was seen with a bridge. In C22, eleven cells, out of 106 examined, showed single bridge chromatids at anaphase I, in all cases apparently unaccompanied by fragments (Fig. 4f). These observations of anaphase bridges and fragments suggest that *P. alpina* (Edin.) and its related hybrid C21 are heterozygous for inverted sections of chromosomes. An analysis of pachytene pairing might have clarified the situation in C22 where all bridges observed were unaccompanied by fragments, but was unfortunately quite impracticable. The inverted regions are probably short, since anaphase bridges were observed in only a small proportion of cells in any sample.

The double bridge at anaphase I in C21 (Fig. 4d) requires the formation of two complementary chiasmata in the inversion loop (Frankel, 1937; Richardson, 1936(a)). Such a distribution of chiasmata is unusual in the plantains as, at diakinesis and metaphase, normal bivalents never had more than one chiasma on the same side of the centromere. Disappearance of chiasmata through terminalization is improbable. It is possible, however, that, with an inversion paired in one arm, special conditions of stress may arise resulting occasionally in a second chiasma. Anticipating, it may also be pointed out that two types of trivalent have been found in triploid hybrids with triple chiasmata which must result from two chiasmata forming in one arm of a chromosome.

Chromatid bridges at anaphase I were found in *P. alpina* (Edin.). Two cells each showed a single bridge with fragment (Fig. 3d) and two others each had a broken bridge without a fragment.

(4) *Meiosis in triploid hybrids.* C37 (*P. alpina* (Edin.) × P81), C38 (*P. alpina* (Edin.) × P84) and C39 (*P. alpina* (Edin.) × P91). As is to be expected, more variation in metaphase association occurred here than in the samples hitherto described. The chromosomes mostly formed trivalents. These were either simple chain or triradiate types with two chiasmata, or a ring and rod type, requiring three chiasmata. In nearly all cells some chromosomes remained as bivalents and unpaired univalents (Fig. 5a, d).

Table 4. *Chiasma frequencies of triploid hybrids*

Sample	No. of cells	No. of $\frac{1}{2}$ Xta per cell							Mean $\frac{1}{2}$ Xta per chromosome
		16	18	20	22	24	26	28	
C37	9	1	1	1	1	3	—	2	1.259
C38	15	1	—	3	3	3	5	—	1.274

The extent of association in individual cells varied from a maximum of six trivalents, observed in a single cell of C38, to six bivalents with six univalents; the average chiasma frequencies for C37 and C38 are given in Table 4.

A peculiar configuration suggesting the occurrence of translocation was observed in one pollen mother cell of C38, where two cells showed a 'ring-rod' trivalent having the 'rod' chromosome terminally associated with the interstitial region of one chromosome



of the 'ring' pair (Fig. 5*b*). As in other triploids, segregation at anaphase I was very irregular. Univalents lagged behind but passed to the poles without dividing (Fig. 5*e, f*).

The following distributions were recorded:

	C37	C38	C39
9:9	3	3	2
9:8 with 1 lagging	3	1	2
9:7 " 2 "	—	—	1
8:8 " 2 "	—	2	1
10:8 " 0 "	1	0	4
10:6 " 2 "	—	—	1
9:6 " 3 "	1	—	—
12:6 " 0 "	1	—	1

In C38 four cells and in C39 two cells each with a single bridge were observed at first anaphase, but in none of these could fragments be found (Fig. 5*c, e*).

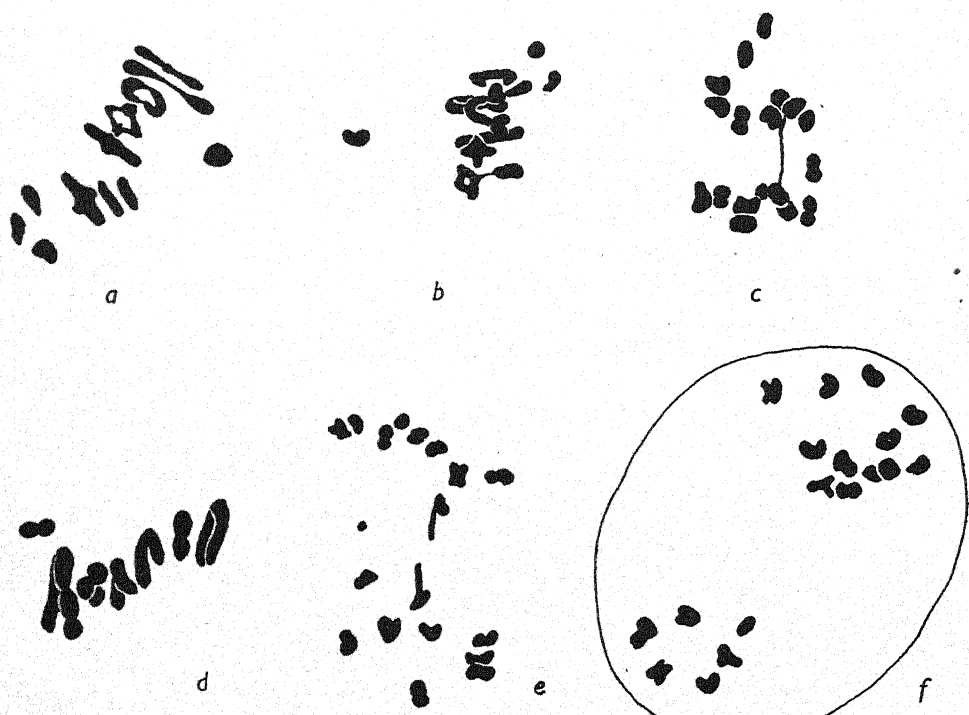


Fig. 5. Triploid hybrids. C37: (a) metaphase; C38: (b) metaphase-translocation in lowest trivalent, (c) anaphase I—bridge; C39: (d) metaphase, (e) anaphase I—bridge and fragment, (f) metaphase II. (All from acetocarmine smears.)

(5) *Meiosis in tetraploid progeny of triploids*. C37 (N.S.) (natural seed off C37) and C38 (N.S.) (natural seed off C38). Despite their inherent sterility a small amount of material was raised from seed harvested off the triploids C37 and C38. These offspring proved to be entirely tetraploid. The metaphase behaviour of both differed in no way from that described for the other tetraploids and the average chiasma frequency for C38 (N.S.), determined from twenty-six cells, was 1.07 chiasmata per potential bivalent. First anaphase was not studied in detail, but one cell was seen in C38 (N.S.) showing a single chromatid bridge unaccompanied by a fragment.

## IV. COMPARATIVE MORPHOLOGY OF ALPINE DIPLOIDS AND TETRAPLOIDS

In order to amplify the cytological study of the diploid-tetraploid relationship, a brief consideration of the salient facts of comparative morphology has been included. The data cited are those of Dr Gregor (unpublished) and refer to Alpine diploid samples P 111, P 116, P 118 and Alpine tetraploid samples P 117, P 119, P 130, P 133, P 62 and P 63. The methods of obtaining the data presented in Table 5 are detailed in Gregor *et al.* (1936).

Table 5. *Mean values of diploids and tetraploids*

Character	No. variates		Mean	
	Diploid	Tetraploid	Diploid	Tetraploid
Leaf length, cm.	81	511	10.75	23.61
Leaf breadth, mm.	81	511	4.98	5.36*
Leaf thickness, mm.	191	519	0.68	0.78
Scape height, in.	185	516	7.60	14.03
Scape length, cm.	187	515	17.89	35.09
Scape thickness, mm.	187	515	1.51	1.63
Bract length, mm.	191	518	2.40	2.99
Sepal length, mm.	191	518	1.95	2.53
Anther length, mm.	189	516	1.93	2.18
Seed length, mm.	260	780	0.85	1.03
Flowering grade	190	520	9.30	5.96
Spike number	80	310	99.21	178.67
Spike density	186	516	17.82	14.71

\* 0.5 level of significance; all other values highly significant.

It will be seen from the table that for all characters there is a difference between the mean values for diploids and tetraploids. With the exception of one character, viz. leaf breadth, the difference is in all cases highly significant. The tetraploids always show the higher mean value, and it is apparent that these figures are an expression of the 'gigas' habit. These comparisons are paralleled by similar measurements for a number of other characters, although it may be noted in passing that preliminary studies have failed to show any difference in the mean size of pollen grains derived from diploid and tetraploid plants.

In garden cultures of sample P 119, a sample predominantly tetraploid, the occasional diploids could be recognized by their smaller size. But it must be emphasized that neither quantitative nor qualitative differences are sufficiently marked to enable Alpine tetraploids to be distinguished with certainty in the wild.

## V. DISCUSSION

(1) *Chiasma frequency*. A considerable amount of data is available as to the frequency of chiasmata in related diploid and tetraploid forms. Upcott (1939), discussing the incidence of chiasmata in *Primula kewensis* (4x) and its diploid progenitors, compared her results with those of nine other similar investigations. It was pointed out that the tetraploid always had a diminished frequency of chiasmata which varied in different species from 98 to 77 % of the frequency in the related diploid. Other similar cases have also been found in tetraploid and diploid *Setcreasea brevifolia* (Richardson, 1936(b)), for example, and in *Tradescantia* species (Anderson & Sax, 1936).

By contrast with these examples a few cases are known of the reverse behaviour. Thus Levan (1937), examining two sister plants from a cross between two varieties of *Allium*

*paniculatum* found one diploid ( $2n=16$ ) with 0.85, and the other tetraploid ( $2n=32$ ) with 0.97 chiasma per chromosome. Also Muntzing (1937) found that the diploid *Dactylis Aschersoniana* ( $2n=14$ ) had  $10.86 \pm 0.15$  chiasmata per cell, whereas the tetraploid *D. glomerata* had  $23.04 \pm 0.46$ .

As regards the *Plantago* types of the present study the summary of chiasma formation in Table 6 shows that the tetraploids have a chiasma frequency closely comparable with that of the diploids. No detailed comparison can be usefully made, since it is not known that any tetraploid is directly derived from any diploid(s). It may be concluded that tetraploidy in these plantains is accompanied by little, if indeed any, decrease in chiasma frequency.

Table 6. *Chiasma frequencies in diploids and tetraploids*

Sample	No. of cells	Total Xta	Xta per cell
Diploid:			
P82	20	132	6.6
PsS9	23	155	6.7
<i>P. carinata</i>	128	819	6.4
C28	125	838	6.7
C29	110	727	6.6
Tetraploid:			
P63	26	337	13.0
<i>P. alpina</i> (Edin.)	54	721	13.4
C21	23	305	13.3
C22	12	162	13.5

(2) *Interrelationship of diploids and tetraploids.* The meiotic behaviour observed in the tetraploids *P. alpina* (Edin.), P63 and P65 does not permit of any conclusion regarding their allo- or autotetraploid constitution. The chromosome pairing observed is intermediate between that expected for extreme allo- and autotetraploids. The formation of one or two quadrivalents in these types, however, does indicate that two distinct sets of four chromosomes are sufficiently homologous to allow of pachytene pairing and chiasma formation, but, because of the lack of visible differentiation between chromosomes, it is impossible to determine whether the same chromosomes always form the quadrivalents. However, the behaviour of the tetraploid hybrid C21 (*P. alpina* (Edin.), tetraploid  $\times$  PsS9, diploid) provided some indirect evidence as to the probable nature of the wild tetraploids. This hybrid must contain one diploid complement, presumably from a diploid pollen grain, of PsS9. It is reasonable to suppose that intramonoploid pairing does not take place in any of the *Plantago* material and consequently two methods of chromosome pairing are possible in C21. The twelve PsS9 chromosomes may form six bivalents and, in that event, the *P. alpina* (Edin.) chromosomes must also form six bivalents. Alternatively, the twelve PsS9 chromosomes may pair with the twelve *P. alpina* (Edin.) chromosomes to form twelve bivalents. Quadrivalents must represent associations of two PsS9 with two *P. alpina* (Edin.) chromosomes. Pairing in the former case could only occur if the twelve *P. alpina* (Edin.) chromosomes comprise two homologous sets of six. In the second case, it may also be inferred that six homologous pairs of *P. alpina* (Edin.) chromosomes are present since they pair with two homologous monoploid sets of PsS9.

Further evidence relative to the homology of the two chromosome sets in the tetraploid may be gathered from a study of the triploid hybrids of *P. alpina* (Edin.), namely C37,

C38 and C39. As reported previously these triploids may all show a number of trivalents, the maximum of six having been observed in C38. Wherever trivalents are formed, two *P. alpina* (Edin.) chromosomes must either pair together directly or through the intermediacy of a third chromosome from the diploid parent. Such behaviour again indicates a high degree of homology between the two sets of six chromosomes in the haploid complement of *P. alpina* (Edin.). The evidence therefore leads to the conclusion that *P. alpina* (Edin.) is an autotetraploid.

The behaviour of the tetraploid hybrid C22 (*P. alpina* (Edin.) × P63) provides a basis for inferring the constitution of the wild-growing tetraploid P63. Chromosome association in this hybrid was perfectly comparable with that of *P. alpina* (Edin.). Since the chromosomes of P63 either pair together or with those of the presumed autotetraploid, *P. alpina* (Edin.), P63 must also be a presumptive autotetraploid. Moreover the autopolyploid nature of the wild tetraploid populations gains further support from the interfertility relationships of the different diploid populations (Gregor, 1939), which indicate an absence of any appreciable differentiation between the diploid complements, such as would be necessary for the production of allotetraploids.

(3) *Tetraploid fertility*. Assuming that the plantain tetraploids are autopolyploids it might with reason have been expected by analogy with previous observations (Darlington, 1928) that their fertility would be less than that of their diploid relatives. Contrary, however, to this expectation Gregor found that the numbers of capsules set and the

Table 7. *Comparative fertility and competitive value of diploids and tetraploids*

Population	Mean				Percentage fertility	Estimated no. of flowers per plant	Estimated no. of seeds per plant	Comparative seed production per plant
	No. of spikes per plant	Spike length, cm.	No. of flowers per cm. of spike	No. of seeds per cm. of spike				
Diploid: P116, P118	99	3.66	17.8	16.0 ± 0.506	90	6,450	5,797	44
Tetraploid: P119, P130, P133	179	6.07	14.7	12.1 ± 0.147	82	15,972	13,147	100

numbers of seeds present in each capsule were as high for *P. alpina* (Edin.) growing under greenhouse conditions as in any of the European diploids. Similarly, tetraploid hybrids (C21 and C46) directly obtained from the respective crosses *P. alpina* (Edin.) tetraploid × PsS9 diploid and P113 diploid × P65 tetraploid, besides the tetraploids (C37 (N.S.) and C38 (N.S.)) arising from the almost completely sterile triploids C37 and C38, were all fertile to a high degree. The wild tetraploids P63, P65 and P117 also exhibited about normal diploid fertility when grown under glass and hand-pollinated. Under garden conditions, however, the relative fertility of diploids and tetraploids was less easily estimated. The flowering period of the tetraploids was later and more prolonged than that of the diploids and seed production tended to decline as the season advanced. Dr Gregor has, however, made an estimate (Table 7) of the fertility of both tetraploids and diploids. It will be seen that, notwithstanding the lower fertility of late spikes, the average seed production of the tetraploid plants was higher than that of diploids of similar geographic source. The fertility of the tetraploids seems almost certainly to be



associated with the low frequency of chiasmata and quadrivalent formation. As a result, meiotic segregation is regular and in the majority of cells twelve chromosomes pass to each pole at first anaphase. The plants are therefore fertile. The behaviour is in keeping with the conclusions of Kostoff (1939) concerning the fertility of polyploid plants with low chiasma frequency.

From the above remarks it may therefore be safely inferred that these apparently autopolyploid populations are not only capable of successful sexual reproduction, but that, in so far as seeding rate determines competitive survival in nature, they are at least as favourably situated as their diploid associates.

(4) *Gene exchange between diploids and tetraploids.* The meiotic behaviour of the diploid hybrids indicates a high degree of chromosome homology and a high potentiality for gene exchange between diploid populations. Whilst the plantains represent one more case where samples of wild populations have been found carrying heterozygous inversions these do not, as yet, seem to have contributed to the formation of barriers to exchange of genes within either the diploid or tetraploid populations. On the other hand, exchange of genes between diploid and tetraploid populations, though possible, must occur very infrequently under natural conditions.

It seems probable that the production of diploid gametes by members of the diploid population is a method whereby the tetraploid population can acquire genes present in the diploid population. For example, in the tetraploid hybrid C21, obtained from the cross *P. alpina* (Edin.) tetraploid  $\times$  PsS9 diploid, it seems that the functional pollen grain from the diploid parent must have carried twelve, instead of the normal six, chromosomes. In contrast to the above example a fertile tetraploid C46 arose from the cross P113 diploid  $\times$  P65 tetraploid, a fact which strongly suggests that, in the wild, exchange of genes could take place as a result of the production by diploids of unreduced egg nuclei. Although no direct evidence was found in either diploid PsS9 or P113 of the production of unreduced gametal nuclei, one pollen mother cell of the diploid hybrid C29 was almost certainly tetraploid. By analogy with normal tetraploid spore mother cells, this might be expected to produce diploid pollen grains.

Gene exchange through the intermediacy of triploids is another possibility. For instance, the tetraploids C37 (N.S.) and C38 (N.S.) originated from the open-pollinated triploids C37 and C38 respectively. Meiosis in these triploids showed that the chromosomes of the tetraploid parent were sufficiently homologous to pair and form chiasmata with chromosomes of the diploids P81, P84 and P91. The derivative tetraploids must contain twelve chromosomes from the triploid parent and twelve from the pollen grains of unknown origin. Vegetatively the triploids are vigorous, but, as was only to be expected from the observed chromosome distributions at anaphase they were almost completely sterile. On the other hand, the few tetraploid plants produced by these triploids were fertile, not only when crossed among themselves, but also when crossed with another tetraploid of wild origin. It is to be expected that in nature tetraploids of this kind are most likely to arise in habitats occupied by a mixed population of diploids and tetraploids. If then, even a single triploid happened by chance to become established and leave tetraploid progeny, the requisite opportunities for the fertilization of such offspring by tetraploids would be available.

While no experimental evidence concerning the origin of tetraploids direct from diploids has so far been obtained it has been possible to demonstrate how the variability

of the former can be enriched in regions where the two populations come into contact with each other. In nature, the production of triploids is likely to be negligible except, perhaps, where self incompatible tetraploids occur in very low frequency among diploids or vice versa. However, the chances of triploids ever establishing themselves in greater concentration than as sporadically occurring individuals are extremely small. Thus in the absence of an intermediate triploid population the line of demarcation between the cytologically stable diploid and tetraploid populations will remain clearly defined. Notwithstanding this lack of an appreciable connecting population the absorption of diploid population genes by the tetraploids can still take place unobtrusively through the agency of diploid gametes. As mentioned in the introduction these two populations were provisionally regarded as belonging to different *coenospecies*, i.e. their status as independent units was recorded as complete. But, in view of the evidence now available, this taxonomic conception is no longer tenable and the true population status of both diploids and tetraploids is more adequately expressed, in terms of population taxonomy, by regarding them as separate *ecospecies* of the same *coenospecies*. In other words the diploid and tetraploid populations constitute two unquestionable units of evolutionary significance having a low potential capacity for the exchange of genes.

#### VI. SUMMARY.

1. The basic chromosome number in the group of plantains allied to *Plantago maritima* L. is  $x=6$ . The North American, Greenlandic and north European populations are  $2x$ ; those inhabiting the European Alpine region are of two kinds (a)  $2x$  and (b)  $4x$ .
2. The  $4x$ , like the  $2x$  populations, form a cytologically stable and intrafertile group. Their fertility is attributed to a low incidence of multivalents.
3. The occurrence of a certain amount of structural hybridity has been inferred from the behaviour of hybrid material.
4. The meiotic behaviour in  $4x$  types and in  $3x$  hybrids between  $4x$  and  $2x$  plants strongly suggests that the  $4x$  types are autotetraploids.
5. The occasional acquisition by the  $4x$  population of genes present in the  $2x$  population has been demonstrated as follows: (1) the fertilization of a  $4x$  plant by a  $2x$  gamete from a  $2x$  plant, (2) the fertilization of a  $2x$  egg nucleus of a  $2x$  plant by a normal  $2x$  gamete of a  $4x$  plant, and (3) the production of  $4x$  zygotes by  $3x$  hybrids.
6. Since both the  $2x$  and  $4x$  populations lead an almost independent evolutionary existence in consequence of their potentially low capacity for gene interchange it is suggested that, taxonomically, they should be regarded as separate *ecospecies* within a common *coenospecies*.

The author wishes to express his indebtedness to Dr E. W. Fenton of the Edinburgh and East of Scotland College of Agriculture for his kindly interest in the cytological work and to Dr Gregor, both for the provision of material and for invaluable help and constructive criticism.

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# A MIDDLE BRONZE AGE PALSTAVE FROM BURIED FOREST AT WOODWALTON FEN, HUNTS

DATA FOR THE STUDY OF POST-GLACIAL HISTORY. VIII

By H. GODWIN, PH.D.

*Botany School, Cambridge*

(With Plate 2 and 3 figures in the text)

## INTRODUCTION

Since the publication of Sir Cyril Fox's admirable *Archaeology of the Cambridge Region* it has been apparent that during middle and late Bronze Age times the margins of the East Anglian fenland were subjected to an intensity of human invasion not equalled at any other time in prehistory. A great many of the extremely abundant bronze objects recovered from our fenlands have come from situations where they were evidently associated with a vigorous development of the so-called 'buried forests'. It has been shown that fen woods of pine, oak and yew are associated with this archaeological period at Wood Fen near Ely (Godwin & Clifford, 1935), at Methwold Fen (Godwin, Clark & Clifford, 1934), and at Woodwalton Fen itself (Godwin & Clifford, 1938). Opportunity to confirm this correlation and to strengthen the correlation of archaeological periods and forest history as shown by pollen analysis, was recently offered to members of the Fenland Research Committee by another discovery from the Fenland margin.

Early in 1942, during the operation of clearing prostrate trees from peat land in Woodwalton Fen, a bronze palstave was discovered, and the find reported to Major G. Fowler, who with the author visited the site on 22 March. The finders were Mr Fuller and Mr J. A. Chance of Woodwalton village, and it appeared that ploughing operations had prevented exact marking of the site or retention in situ of the axe or tree with which it was associated. The site was in the south-west corner of the 13-acre field east of Castle Hill Farm, and about 1000 ft. (320 m.) south by east of the farm (6 in. O.S. 1926 Hunts, Sheet XIV, N.W.).

## TYPOLGY

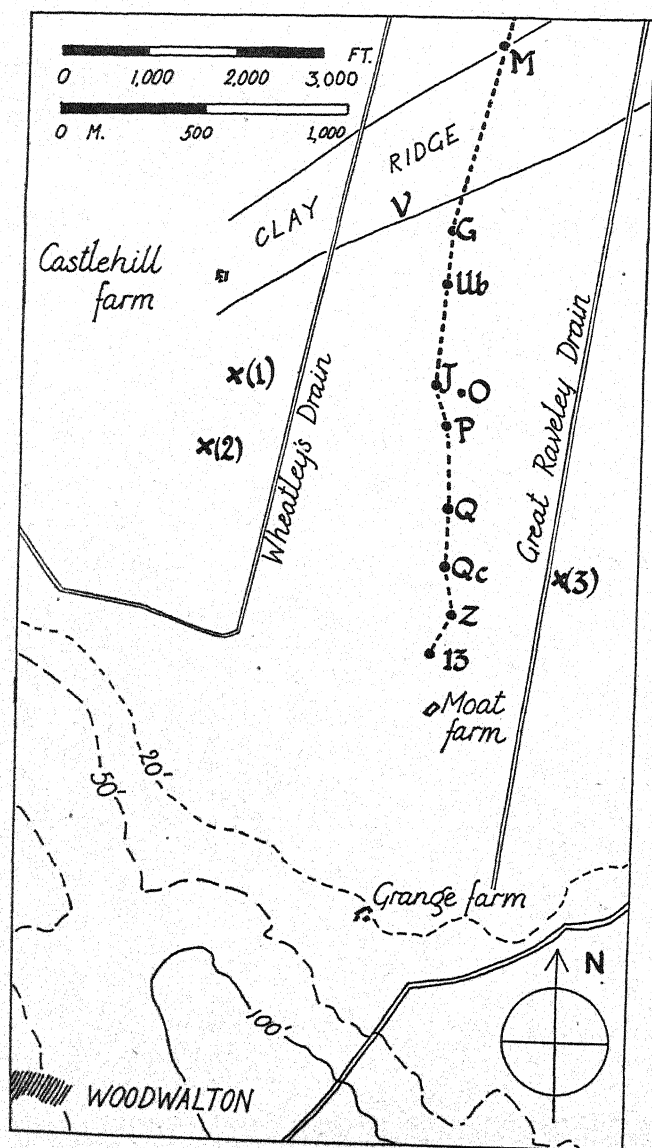
The palstave (see Pl. 2) is a common middle Bronze Age type with a large stop-ridge and side-flanges. A low stirrup-shaped ridge continues from the flanges to the flat surface of the blade, enclosing a shield-shaped area in the manner said by Fox (1923) to be characteristic of palstaves of the Cambridge region. From the apex of the loop a faint central ridge runs farther down the centre of the blade. Palstaves of this type have been found in several hoards, e.g. Birchington and Burham.

## PROVENANCE

It was reported that the axe was found sticking into the trunk of a prostrate oak tree some 30 ft. in length and 10 in. to 1 ft. in diameter at its base. The blade of the axe was parallel with the axis of the tree, about 6 ft. 6 in. from the base of the trunk, and

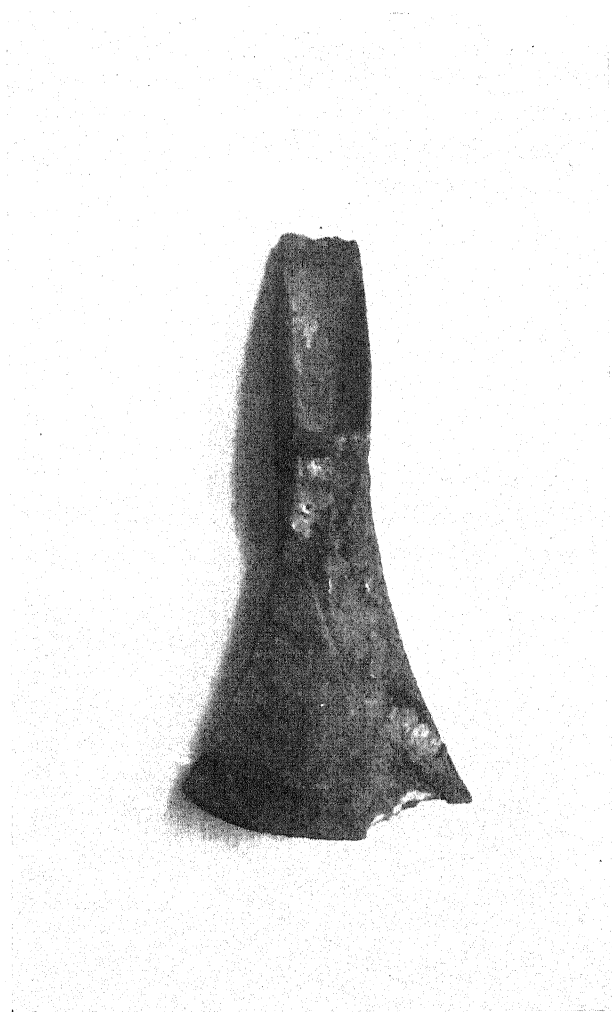


embedded only by one corner of the blade as if it had been pecked into the tree for the time being and then forgotten, at a time when the tree was still standing. Slight damage to one corner of the axe blade indicates the truth of this account. The axe was dislodged



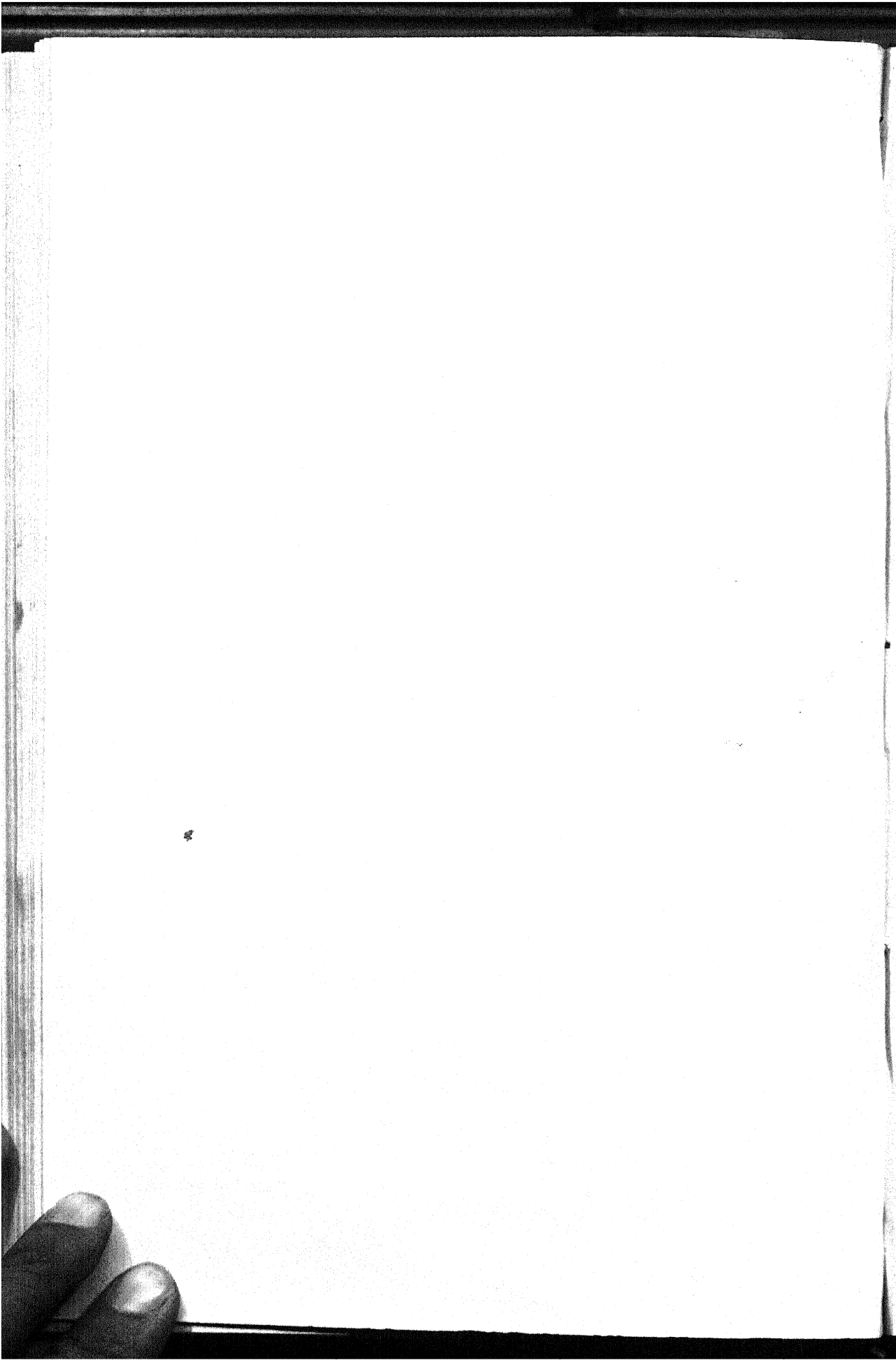
Text-fig. 1. Map of the fen margin at Woodwalton, Hunts, showing sites of (1) middle Bronze Age palstave, (2) socketed adze of the late Bronze Age (Garrood, 1929), (3) early middle Bronze Age palstave (Garrood, 1930). The heavy dotted line indicates the position of the long section established by borings and excavations across Woodwalton Fen (Godwin & Clifford, 1938). The edge of the peat at the fen margin lies just north of the 20 ft. contour.

from the tree by the shovel of the finder. It was said that the axe was at a depth of about 2 ft. 6 in. from the surface (76 cm.), but it was also added that the tree roots just projected into the ploughed soil, and this being about 10 in. (25 cm.) in depth, with the



Middle Bronze Age palstave from Castle Hill Farm,  
Woodwalton, Hunts.

GODWIN—A MIDDLE BRONZE AGE PALSTAVE



tree thickness of 10 in. to 1 ft. (25-30 cm.), it seemed reasonable to suppose the base of the tree trunk was about 1 ft. 8 in. (50 cm.) down from the surface. An excavation as near as could be guessed to the site of the find gave the following stratigraphy:

0-25 cm. Ploughed black peat soil, locally with remains of sedge (*Carex*).

25-70 cm. Undisturbed fen-wood peat, with abundant bark and twigs of alder (*Alnus*) and fine sedge roots. At 48 cm. oak (*Quercus*) wood was encountered.

70-80 cm. Transition to underlying sandy clay with frequent small flints (boulder clay).

From this excavation samples for pollen analysis were taken at intervals of 5 cm.

The field was densely littered with the remains of trunks and stubs of excavated trees, and there were numbers of trunks in the farmyard. The bulk was alder (*Alnus*), but there was also much oak (*Quercus*), some pine (*Pinus*) and some slender yew (*Taxus*) about 3-4 in. in diameter. All save the alder were uniformly straight unbranched trunks clearly grown in good forest stand, and this was true for the oak in which the axe was found. Some of these forest trees were rooted in the boulder clay but others were grown on the peat itself.

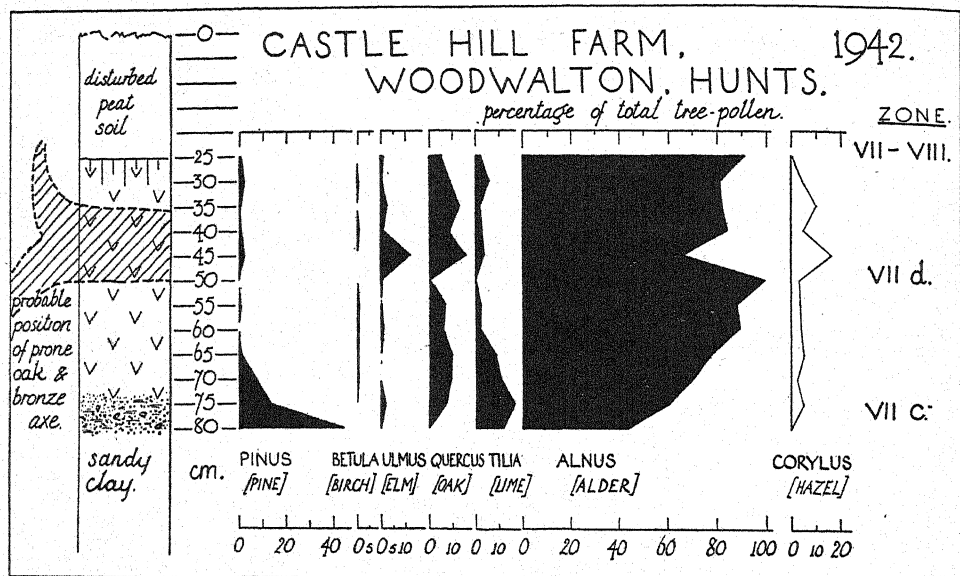
It fortunately happens that the peat stratigraphy of this portion of the fenland margin has already been investigated in detail (Godwin & Clifford, 1938) and a complete profile established by borings and excavations from the edge of the peat northwards through Woodwalton Fen. The line of this profile is shown in Text-fig. 1. From this it will be apparent that the axe site, like two other Bronze Age sites recorded nearby, lies to the south of a prominent ridge of clay which crosses Woodwalton Fen at or near the ground surface, and encloses a shallow basin between itself and the upland. The published investigations have shown that this marginal basin was filled to a maximum depth of about 7 ft. (200 cm.) with peat. This peat is wood peat for about the lower two-thirds of the profile; in it are found very abundant and substantial tree-remains. Thus a large pine stub was excavated at Ub, and much alder and willow wood was recorded elsewhere. Above the wood peat was a layer of raised-bog sphagnum peat, a development exactly parallel with that in the fen to the north of the clay ridge, and one reflecting a sharp alteration in vegetation and in climate. This sphagnum peat was shown to extend very far towards the fen margin, but in the cultivated peat soil of the field where the axe was discovered no trace of such peat could be found: it seems probable that, although once there, it has been removed by cultivation. This development of raised-bog peat has been taken to represent the subatlantic climatic deterioration which corresponds with the beginning of the Early Iron Age, and if our assumptions are correct the wood peat in our profile must, on stratigraphic grounds, predate this.

#### POLLEN ANALYSES

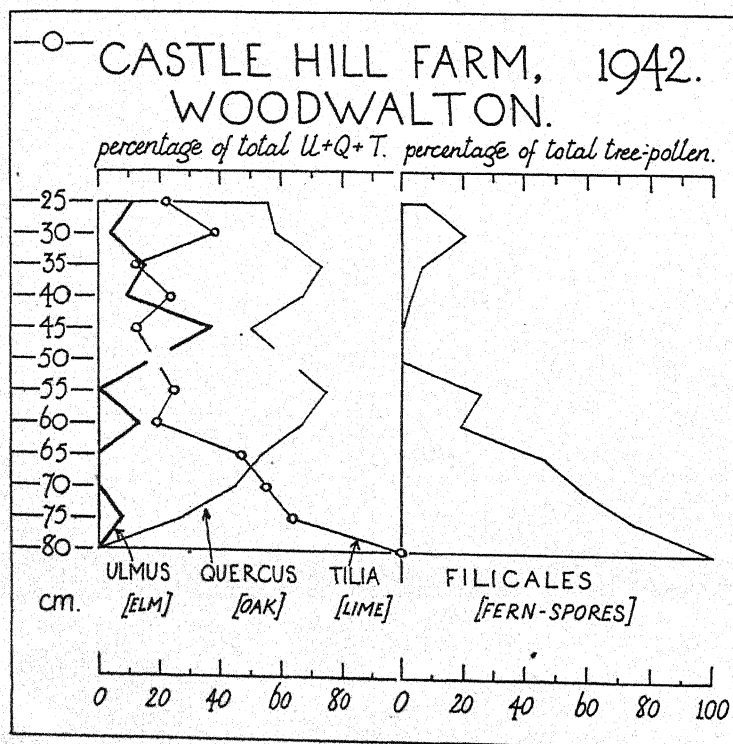
Fortunately to supplement this deduction we can bring the results of pollen analysis at the site of our profile, and can compare these with pollen analyses from other sites close by, namely Woodwalton Ub, and Woodwalton A (on the profile mentioned), Ugg Mere F, and Trundle Mere B. The pollen diagrams are given in Text-figs. 2 and 3.

As in the diagrams from Woodwalton A, and Woodwalton Ub, it is at once clear from Text-fig. 2 that local influences predominate throughout, those of local pine at the base and of alder thence to the top. The pollen of trees from the upland is correspondingly reduced in amount and interpretation of the diagram correspondingly more difficult. However, by expressing *Quercus*, *Ulmus*, and *Tilia* as percentages of the total which





Text-fig. 2. Pollen diagram from middle Bronze Age palstave site at Castle Hill Farm, Woodwalton. The influence of the local fen woods, chiefly alder, predominates throughout. The V marking in the profile indicates wood peat, and the vertical shading at the top, sedge peat coming in.



Text-fig. 3. Left: Oak, elm and lime pollen treated independently of other tree pollen, pollen of each genus expressed as a percentage of the sum of the three. Right: Fern spores expressed as a percentage of total tree pollen.

they jointly make, it is possible to relate this to the earlier series (Text-fig. 3). *Tilia* is strongly preponderant at the base, but diminishes upwards, becoming more or less of the same order as *Ulmus* in the upper half of the profile. This supports the reference of the greater part if not the whole of the diagram to zone VII<sub>d</sub> of the Fenland series. Zone VII-VIII can hardly be represented since *Tilia* is still present in fair amount at the top of the series, *Betula* is still very low, and there is no trace of *Fagus* or *Carpinus*. This agrees with the stratigraphic evidence already mentioned. Zone VII<sub>c</sub> in the Fenland sequences corresponds with the period of deposition of the fen clay, the edge of which lies 1¼ miles (2 km.) to the north of the axe site, beyond the hard clay ridge. The fen clay does not penetrate into the marginal basin, but earlier evidence demonstrated that pine trees were growing on the peat surface both before and after the fen clay deposition. The non-tree pollen was carefully examined in the peat samples, but nothing in it suggests any evidence of a stage of increased wetness having affected this marginal position. The curve for fern-spore frequency given in Text-fig. 3 probably indicates a local change in the density of fern undergrowth in the fen woods throughout the series.

In comparing this Castle Hill Farm diagram with that from Woodwalton Ub, it should be remembered that the latter is a longer series (230 cm.) from the deepest part of the marginal basin, whilst the former is only short (80 cm.), and coming from the edge of the basin deposits probably contains peat formed over a shorter period. Although the axe site was not levelled, reference to the published section suggests that peat as shallow as we find there probably first formed *after* a good deal of peat formation had already gone on at Ub. This accords with the pollen-zonation proposed and with the age of associated Bronze Age finds.

#### ASSOCIATED FINDS

The map (Text-fig. 1) indicates the position of two discoveries reported close to the axe site. The first, marked (2), was a late Bronze Age socketed adze with a loop set at right angles to the plane of the blade found lying on a 'bog-oak' at Castle Hill Farm and only about 860 ft. (260 m.) to the south of the axe site (Garrood, 1929). It appeared on enquiry that the axe was ploughed up from a depth of about 15 in. (38 cm.).

The second find, marked (3) in Text-fig. 1, was an early middle Bronze Age palstave with infolded flanges, discovered on the east side of the Raveley Drain among the roots of a 'bog-oak' between 3 ft. 6 in. and 4 ft. (106-122 cm.) from the surface (Garrood, 1930).

It will be noted that all three bronze finds have been associated with buried trees, and that all lie in the same marginal fen basin, which evidently in middle and late Bronze Age times was covered with fen woodland quite accessible and apparently quite attractive to men of that time. It is perhaps surprising that cut wood has not yet been reported here.

#### SUMMARY

The discovery of a middle Bronze Age palstave at this marginal fen site has strengthened the correlation between this archaeological period and (a) the development on fenland margins of fen woods of alder, oak, pine and yew, (b) the termination of zone VII in the pollen analysis zonation for Fenland and British forest history.

The author wishes to thank Major G. Fowler for his very kind co-operation in the field work, his notification of the discovery in the first instance, and for the photography of the axe.

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# CONTRIBUTIONS TO OUR KNOWLEDGE OF SAPROPHYTIC ALGAE AND FLAGELLATA<sup>1</sup>

## III. *ASTASIA*, *DISTIGMA*, *MENOIDIUM* AND *RHABDOMONAS*

By E. G. PRINGSHEIM

*Queen Mary College, London University, Department of Botany*

(With 20 figures in the text)

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### I. INTRODUCTION

In the first communication of this series it was shown that various saprophytic Flagellata devoid of chlorophyll are capable of multiplication in suitable putrefaction cultures in the dark. Among them the colourless saprophytic Euglenineae, called Astasiaceae, are especially common in cultures inoculated with mud from the bottom of polytrophic waters.

The conditions of life within such enriched cultures are as yet very unclear. They contain mixtures of diverse micro-organisms differing widely from one another. From them it is, however, possible to obtain secondary cultures which contain only a single species of Flagellata. Further subcultures can be effected with a great measure of positive success, so that Euglenineae can be raised in quantity and a supply maintained.

Unfortunately, it has hitherto proved impossible to obtain multiplication of the majority of Astasiaceae except through the agency of putrefaction cultures. Two species only, *Astasia (ocellata) quartana* and *A. (Chattonii) longa*, have so far been grown in pure culture. The methods, which were so successful with these two species, failed with all the others, though up to twenty-five species with more than forty strains are involved.

<sup>1</sup> The two earlier communications were published in *Archiv für Protistenkunde* (Pringsheim, 1936, 1937a).



## II. THE MODE OF OBTAINING CLONE CULTURES

In a mixture of unicellular algae it is often difficult to refer individuals to definite species. This is due to the fact that shape and internal structure are influenced by the environment. The difficulty is especially great in the genera *Astasia* and *Distigma*. They undergo metabolic changes of shape in a very short time.<sup>1</sup> An adequate diagnosis therefore cannot be made without describing the various forms the cell can have. Metaboly especially occurs when conditions suddenly change as is the case in mounting a microscopical preparation. They mainly consist in contractions, twisting and sticking to solid particles or the surface of the glass. If the cells are allowed to swim freely under constant conditions cells straighten and metaboly disappears. But this state of 'normal' shape may never be resumed under a cover slip. We therefore have to watch the cells in a hanging drop or in an open preparation on a slide for more than a few minutes till they are again in an undisturbed, physiologically balanced, state.

An even greater difficulty arises from the fact that the shape eventually adopted by a certain species is not the same under all circumstances but has some, so far unknown, bearing on the conditions of the medium. As a rule all individuals are influenced in the same way. That is why mistakes may easily be made.<sup>2</sup> It takes a long time to watch a strain under various circumstances to become sure of specific differences and identities. Therefore culturing is indispensable for systematic work in this group.

To prepare adequate diagnoses, cultures of course should originate from single cells and show extensive multiplication. The procuring of such clone cultures to begin with proved difficult (Pringsheim, 1936, p. 48). Single cells transferred to fresh putrefaction tubes showed no multiplication, although ten or more cells did.

It was found, however, that this difficulty could be overcome by improving my putrefaction culture method. In most cases starch provides the best organic material. This is mixed with a little soil and chalk, and a small quantity is put on the bottoms of test-tubes. Then these are filled to a height of about 4 cm. with ordinary garden soil not too rich in decaying organic residues, usually called humus, and containing a good quantity of sand. Tap water is added to about 4 cm. above the surface of the earth. The tubes are provided with cotton-wool plugs and heated for about half an hour in a steam chamber. When the tubes are inoculated, sterile water can be added at convenience. It is not advisable to fill the tubes to a high level before pasteurizing, because stoppers would be soiled by rising soil particles during heating. Such tubes are best inoculated a few days after heating, but they can be used even weeks later.

When this procedure was followed almost every uniflagellate culture was successful, though sometimes four or more weeks elapsed before a copious development was observable at room temperature. As a putrefying material plain starch usually was best. Sometimes however starch-containing seeds, such as cereal grains, were used with better results.

With the help of these methods a number of Astasiaceae, in part new, were raised from diverse materials. Usually, however, there appeared certain earlier described species such

<sup>1</sup> Metabolic changes occur not only in green and colourless Euglenineae but also in Chrysomonadineae and other Flagellata as well as in ciliates. But they never reach a degree, making identification very hard, as they do in the case of the Astasiaceae.

<sup>2</sup> I think there are no other organisms which undergo as large changes according to living conditions as do metabolic Euglenineae.

as *Rhabdomonas costata* (syn. *Menoidium longum*), *Distigma proteus*, and a number of small *Astasia*s; but it is probable that numerous other unknown or insufficiently described species could be obtained from other habitats. This is suggested by the rich collections of interesting forms found in certain places, such as the polluted pasture pools above Lunz in Austria or the moorland margins of the Pflegersee in Bavaria.

### III. GENERIC AND SPECIFIC CHARACTERS AMONG ASTASIACEAE

All experts are nowadays of the opinion that the saprophytic Euglenineae have descended from green ancestors. In some cases this relation is still perfectly clear, and it is possible to specify the near allies of the apochlorotic forms among green species. In other cases this is by no means easy. One difficulty in relation to this conception of affinity lies in the fact that although all green genera possess colourless members, the opposite is not true of all the saprotrophic ones, and still less so for the holozoic ones.

The genus *Euglena*, with its diversity of shape and ecological needs, is of special interest in this connexion. *Astasia* in many members is so closely related to it that certain colourless species have with justification been described as apochlorotic varieties of green species of *Euglena*. Furthermore, *E. gracilis* is known to be distinguished only with difficulty from an *Astasia* when grown in complete darkness (Ternetz, 1912). Klebs (1883) observed apochlorotic forms of *Euglena acus*, and other authors corroborate this. The same is true of *Phacus* and *Trachelomonas*. The systematic position of all these forms is quite clear. That of *Menoidium*, on the contrary, is rather dubious and at any rate peculiar. As I emphasized earlier (1936, p. 57) this genus is related both to *Lepocinclis* and to *Phacus*. Furthermore, the genus *Menoidium* is not homogeneous, so that nothing which would be generally valid can be said as to its affinities.

A relation between the biflagellate colourless *Distigma* on the one hand and the green genera *Eutreptia* and *Gymnastica* (syn. *Eutreptiella*) on the other, has been assumed (Pringsheim, 1936, p. 84). Features common to both the pigmented and the colourless species are: an often extremely high degree of metaboly, exceeding that found in any species of *Euglena* or *Astasia*; two flagella, serving for movement and apparently always different from one another, a feature found in no other member of Euglenineae; small paramylon grains.

It is not easy to select the right specific features for *Astasia* and *Distigma*, because high modifiability and metaboly and the difficulty of fixing the cells for observation render it hard to determine the limits of species. Specific definition has become increasingly difficult as more species have been detected. Without living cultures available for comparison, certain of the newly described species could not with certainty have been recognized as different. I propose to discuss the characters in the same order as in the first communication (1936, p. 61 seq.):

(1) New names had mostly to be given to the species, partly because no known species conformed to the type studied, partly because the earlier descriptions were no longer adequate after a number of further species had been found.

(2) Earlier drawings are in part no longer sufficient. This is specially true of those species which are liable to change of shape, and which can only be satisfactorily characterized by several drawings. New illustrations had therefore to be prepared. Outline drawings, including paramylon grains, were considered to be sufficient.

(3) The shape of the body is best described from undisturbed, free-swimming individuals, because these may be considered as normal and characteristic. It is therefore necessary to

employ open drops of large size which allow only the use of weak objectives. The changes of shape, spoken of as metaboly, are likewise important in the definition of species, since they are represented in quite different degrees and afford varied pictures. The strongest, 'convulsive' contractions, which often are associated with the throwing off of the flagellum, seem for the most part to be called forth by chemical disturbances. In the rigid species this corresponds to a mere loss of the power of movement. In what way a new flagellum is formed is not known. Such seemingly badly damaged individuals can, however, sometimes be used for starting new clone cultures.

(4) The *size* of the body should be measured in living cells exhibiting the greatest degree of extension, which is usually impossible owing to movement. If fixed material is used, the cells must be in an undisturbed state before fixation. It happened repeatedly that a newly discovered strain agreed quite well with the earlier diagnosis of the species, but differed in size. In such cases a new variety had to be created, though the earlier described species may be found again and may differ in some essential features.

(5) The *structure of the periplast* is not always readily detected, but striations following a more or less spiral course appear never to be lacking. They were found even in *Astasia longa* mihi, the only species in which the membrane had been described as smooth in the first communication (Pringsheim, 1936, p. 66), by covering fixed and dried cells with a film of collargol or other colloidal stains. The value of the striation as a specific feature is greatly diminished by the fact that it may be developed to a varied extent in different individuals of the same clone culture. Similar observations have been published by Deflandre (1924), Lefèvre (1931) and Conrad (1934, p. 203). The cause of the phenomenon requires special research.

(6) All statements as to the *length of the flagellum* are to be used with caution. It is impossible to measure it, when the flagellum is undulated or forms ringlets. The only species in which flagella are sometimes straight after death belong to the genera *Menoidium* and *Rhabdomonas*. No remedy for this drawback could be found. I wonder how the precise data in literature as to the length of the flagella have been obtained. Actually we are compelled to undertake rough estimations which, as usual, are related to the length of the body.<sup>1</sup> Among metabolic species an additional source of error lies in the variability of cell length. It is thereby in most cases not possible to say more than that the flagellum is about as long as the body or appreciably longer or shorter. In addition, the length of flagella varies in relation to conditions. It is therefore no reliable specific feature in some cases.

(7) The *eye-spot* is lacking in all the species newly described in this paper. Colourless Euglenineae with eye-spots will be the topic of a further communication.

(8) The position of the *nucleus* is not always the same in a given species or even individual. In some of the species it tends to be almost central, while in others it is situated rather far towards the back end of the cell. The degree of its distinctness varies also. In some species it is evident even in living cells and is provided with a distinct caryosome, while in others it cannot be found without fixation and staining, its position being disclosed only by an area free from paramylon grains.

(9) The grains of *paramylon* often supply important diagnostic features, but these are not so easily used as statements in previous papers would indicate. Paramylon is certainly a reserve substance, although it does not disappear as readily as a result of starvation as starch mostly does. Shape and size of the grains are very variable. During rapid development there are only small quantities of paramylon in the cells, but with slowing down of division it increases in abundance. In old cultures it often disappears again more or less. In certain species paramylon is chiefly deposited in definite places in the cell. Thus, in many species of *Astasia* and *Distigma* most of it is usually found in the anterior part of the body.

The individual granules are approximately cylindrical. The ratio of length to breadth, however, varies so that the actual shape ranges from nearly discoid to rod-shaped or even acicular. Such differences are characteristic of different species. The readiness with which stratification is discernible is likewise a specific feature, not depending only on the size of the

<sup>1</sup> I had the impression that the lengths of flagella are not always quite as diverse as the sizes of bodies, so that larger varieties or modifications have a relatively shorter flagellum than shorter ones.



granules, although in a given species the largest grains show it most distinctly; it depends on unknown properties of the species. If the granules are densely packed they assume a polyhedral shape, but flattening is not the result of direct contact, since narrow interspaces are always recognizable.

Some species, in which metaboly is most pronounced, have very small granules throughout, while large, ring- or rod-shaped paramylon grains are found only in those species which are nearly or completely rigid. This relation can be understood on mechanical grounds. First, a cell with long paramylon rods would be unable to perform strong metabolic movements, and secondly, deposition must be interfered with by such movements, just as in the case of crystallization from a solution.

(10) The manner of swimming in different species merits considerable attention. It affords valuable features, especially in the metabolic species and in those possessing two flagella.

(11) Certain similarities between the species become the more obvious the more species are discovered. They go so far that original diagnoses of species often fail to be unequivocal, and that comparison of species under various conditions are indispensable for identification.

(12) As regards the ecological conditions and the habitats in which individual species thrive, no certain statements can as yet be made; therefore the places only, from which my strains originated, are mentioned.

(13) The composition of the putrefaction tubes for culturing the various species was almost monotonous. Some remarks concerning them are found in the single sections dealing with species.

#### IV. THE GENUS *ASTASIA*

##### (1) General discussion

The genus *Astasia* includes all colourless uniflagellate Euglenineae capable of change of shape or, what amounts to the same thing, the apochlorotic forms parallel to *Euglena*. It comprises also those species which are not capable of performing active metabolic changes, but at most exhibit curvatures of the body, such as *Astasia linealis*, which in this respect as in others belongs to the morphological group of *Euglena acus* (Pringsheim, 1936, p. 78).

The association of certain *Astasias* as apochlorotic parallels to species of *Euglena* is impracticable in most cases, because chloroplasts and pyrenoids afford diagnostic characters in the latter which are not available in *Astasia*. The phylogenetic loss of these characteristics, which is related functionally to the change in the mode of nutrition, results in the absence of features which are indispensable for establishing the true relationship. A system suitable for practical use cannot therefore reproduce the pedigree, until some substitute of the nature of other characteristics has been found. Possibly serological reactions might afford the necessary data.

##### (2) New and imperfectly described species

The following descriptions are more detailed than usual, because identification would otherwise be impossible.

##### 1. *Astasia applanata* n.sp. (Fig. 1)

(1) Free-swimming individuals club-shaped, usually not circular in cross-section but more or less flattened (hence the specific name) or nearly spoon-shaped, with slight torsion. Stronger twists with spiral edges not observed. When disturbed, rather easily assuming a completely flattened, nearly plane shape, the outline becoming approximately trapeziform or like a slightly drawn out lemon. More marked metaboly only



after considerable injury, e.g. the action of poisonous substances, which also leads to cessation of the swimming movement. Under such circumstances the cell assumes the form of a squat bottle or a broad turnip, with accumulation of the protoplasm at the back end and indication of an anterior neck. The front end is never acute or truncate; the gullet and vacuolar system are difficult to see.

(2) Length of body up to  $40\mu$ , the breadth then being about  $10\mu$ .

(3) Distinct close striation, markedly oblique also on parts that are drawn out in a cylindrical manner, in club-shaped parts often running nearly transverse near the anterior end.

(4) Flagellum of about the length of the body.

(5) Nucleus mostly behind, usually not far from the centre, but sometimes near the posterior end. Its situation may change in the same cell with the degree of metaboly. The nucleus is not easily recognizable during life.

(6) Paramylon grains of medium size, discoid or rod-shaped, sometimes like an elongate ring. A few larger ones may occur.

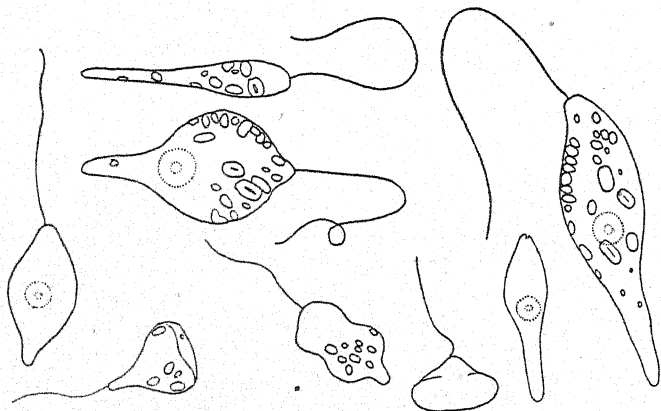


Fig. 1.<sup>1</sup> *Astasia applanata* n.sp. In the left upper corner a cell viewed from the narrow and broad sides respectively showing the flattening typical of the species. The large individual on the right shows the shape, with moderate flattening, mostly seen during swimming.

(7) The motion during free swimming shows no characteristic features, but when progression is hampered the movement is often vibratory, although no secondary flagellum is present (cf. 1936, p. 76).

(8) There is a certain similarity to *A. Dangeardii* Lemm. The strain of this species, which was observed and determined according to the diagnosis of Lemmermann (1913), shows cells which differ from those of *A. applanata* in generally being curved as a whole and truncate at the front end. The description by Lemmermann does not, however, render a strict decision possible. *A. inflata* Klebs must also be taken into consideration; certain greatly shortened and flattened metabolic stages of *A. applanata* fit Lemmermann's diagnosis. The species, which I previously described by this name and which did not, owing to the smaller size of the body,<sup>2</sup> quite agree with that of Klebs, differs

<sup>1</sup> All the figures are drawn to the same scale, the larger ones magnified 1000, the smaller ones 500 times. They are prepared so far as possible from living cells and for this reason they have been kept simple.

<sup>2</sup> Skuja (1939, p. 106) describes and figures a strain of *A. inflata*, which is in good agreement with those of Klebs and Lemmermann. He is of the opinion that it differs from the strain I described, but there seems to be no other difference than that of size.

from *A. applanata* in the curved shape assumed when swimming freely, the smaller dimensions, the greater tendency for metaboly, quicker movement and relatively large paramylon rods (cf. 1936, p. 62).

(9) The strain was reared from material from a polluted upland pool on the Dürrenberg above Lunz.

(10) Good cultures were obtained only with putrefaction tubes containing wheat grains and soil or peat, to which a little  $\text{CaCO}_3$  was added, not with pure starch, as in the other species.

## 2. *Astasia clava* n.sp (Fig. 2)

(1) Body club-shaped when free-swimming. Hence the specific name. Mostly stout, but sometimes slender, elongate and about the shape of a carrot, not curved, flattened or twisted. Metaboly slight, although inflations occur, sometimes one, sometimes two of them with an intervening constriction.

The posterior end can become rod-shaped; the anterior end rarely exhibits this form. The structure of the anterior end could not be exactly elucidated owing to the small size of the cells, but there is a shallow emargination where the flagellum arises; this is situated a little to one side so that the cell is usually somewhat oblique at the front end.

(2) Length of the cell 20–25  $\mu$ .

(3) Striation exceedingly delicate.

(4) Flagellum about  $1\frac{1}{2}$  times as long as the body.

(5) Nucleus only discernible during life by the clear space left between the paramylon grains, situated behind the middle.

(6) Paramylon grains usually all of almost the same size; stratified and specially large grains are lacking. The grains are rod-shaped or polygonal. They often are almost or entirely wanting in the posterior end of the cell.

(7) Swimming movement not very rapid. The cell is mostly not distinctly curved, but is often somewhat irregular in outline so that the spiral path becomes evident.

(8) There is a certain similarity with *A. gomphonema* n.sp., which is alike in the nucleus being situated behind the middle and in possessing a club-shaped body, often with a waist. Differences exist in the finer striation, the absence of torsions and in the greater length of the flagellum in *A. clava*. Compared with *A. inflata* Klebs., *A. clava* n.sp. has much less marked capacity for change of shape, no flattening and more delicate striation.

(9) The material came from the 'Soos' of Franzensbad in Bohemia.

(10) This species multiplied in all kinds of putrefaction cultures.

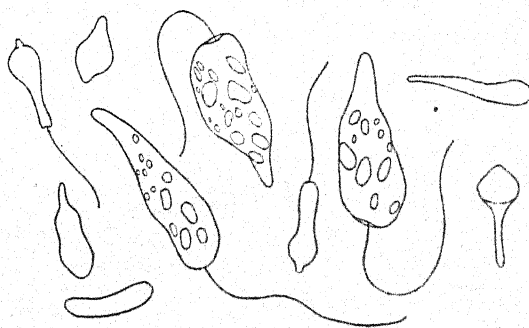


Fig. 2. *Astasia clava* n.sp. The nucleus is not visible in the living cell and therefore not shown.

## 3. *Astasia comma* n.sp. (Fig. 3)

(1) In the free-swimming condition generally like a curved club or a comma (hence the specific name), sometimes distinctly spiral so that when viewed from above the organism appears to bend to and fro. More rarely the body has the shape of a straight club.

Metaboly considerable. Torsions and flattenings may occur but spherical metaboly seems to be absent. Anterior end often somewhat truncate.

- (2) Length  $28-32\mu$ , breadth  $7-8\mu$ .
- (3) Striation delicate but distinct.
- (4) Flagellum of about the same length as the body.
- (5) Nucleus in the posterior end of the dilated part where it begins to taper off conically so that it appears like a sphere lying in a funnel. Both it and the caryosome are rather easily discernible.

(6) Paramylon in grains of different sizes, minute ones behind, and larger ones (often distinct short rods) in front of the nucleus, the largest sometimes with stratification.

(7) Movement during swimming obviously rotatory owing to the curvature of the body, often in wide spirals, sometimes rather irregular and as it were tentative.

(8) This strain is related to my strain of *A. inflata*, but it is seldom completely flattened and is somewhat larger, though not as large as the form described by Klebs under this name, which, moreover, is said to possess a central nucleus.

My strain of *A. inflata* differs also in the relatively shorter flagellum and in the fact that the nucleus is more difficult to distinguish.

(9) Material from the shores of the Obersee above Lunz between decaying leaves of *Carex*.

(10) Thrives very well and reliably in different putrefaction mixtures, with material rich in starch and covered by soil.

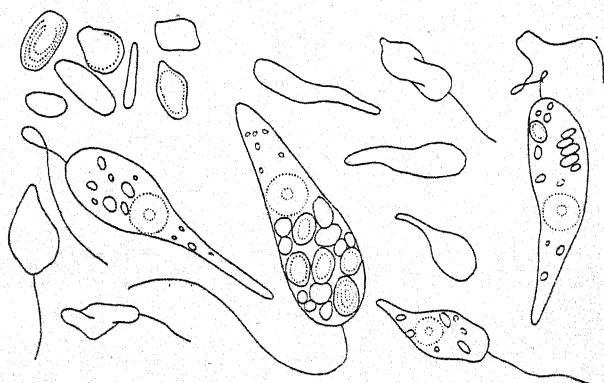


Fig. 3. *Astasia comma* n.sp. On the left an individual showing an unusual degree of extension and with few paramylon grains. Above, a number of the latter, magnified 2000 times, showing their special features.

#### 4. *Astasia cylindrica* n.sp. (Fig. 4)

(1) While swimming freely and undisturbed the cell is obliquely club-shaped, the anterior part being remarkably large and ovoid or spherical, while the posterior end is relatively small and not distinctly demarcated as a process. The metaboly consists mostly in a shortening and broadening of the posterior part, as a result of which the cell becomes distinctly cylindrical. In this state both ends are rounded, the cell remaining straight or being very slightly curved. This characteristic feature provides the basis for the specific name. True ventricose swellings are not produced, although the middle part is sometimes a little inflated.

The cytoplasm appears transparent, the cell having a remarkably delicate appearance. The shape is very variable, but neither flattening nor spherical metaboly nor torsions were observed; at the most there is constriction at the middle which presupposes a slight torsion, resulting in the 'violin'-like shape, which is characteristic for this and several other species of *Astasia*, especially *A. inflata*.

- (2) Length  $16-20\mu$ , when fully stretched up to  $25\mu$ .

(3) Strong spiral striation which is not always easily seen in every cell of a culture, but which is generally distinct.

(4) Flagellum about as long as the body or a little longer. On treatment with alkali or iodine vapour it is shed, but not during fixation with osmic or acetic acids.

(5) Paramylon grains small, elongate-rounded to short rod-shaped. Bigger grains and such as are stratified were not observed.

(6) The path during swimming is generally distinctly spiral owing to the torsion of the body. The movement is rapid and erratic; since its direction is often changed, wide sinuous curves are commonly described.

(7) The new species resembles *A. inflata* var. *minor* (Pringsheim, 1936, p. 62), in which, however, the nucleus is situated farther back and the cell is flattened strongly during metaboly. The typical *A. inflata*, as described and figured by Lemmermann and by Skvortzow, is double as large. *A. clava* n.sp. also shows a certain resemblance to *A. cylindrica*, but it is not curved, possesses more delicate striation, has a longer flagellum

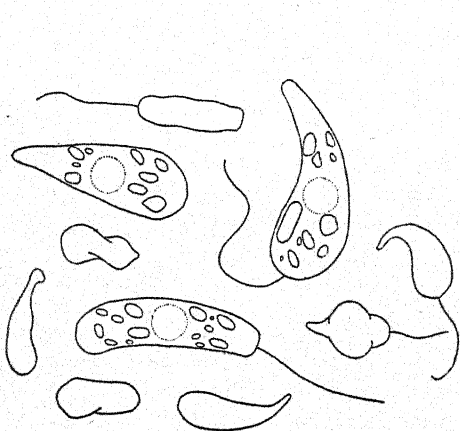


Fig. 4. *Astasia cylindrica* n.sp. The shape to which the specific name refers is only represented in two individuals in order to be able to illustrate the manifold other forms that are assumed. Here and in all the other figures the relative numbers of the different forms cannot be recognized in the figures.

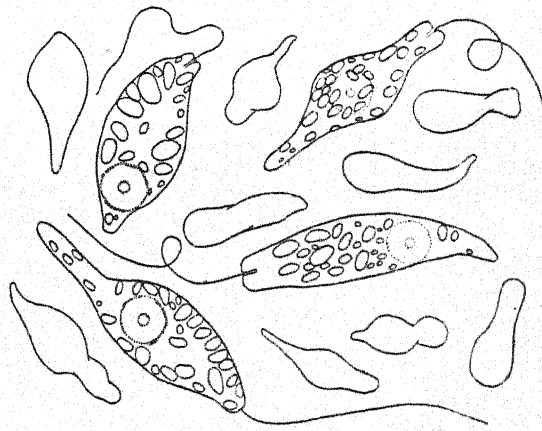


Fig. 5. *Astasia Dangeardii* Lemmermann. The figure of Lemmermann is not very characteristic, so that a comparison does not show whether the forms are identical.

and usually a posterior process distinctly demarcated from the body. Lastly there is also similarity to *A. comma* n.sp., in which, however, the anterior portion is more elongate, while thickening of the posterior part leading to a general cylindrical shape does not occur. On the contrary, the cells of *A. comma* usually remain narrowly elongate, the posterior process disappearing only when the cell is strongly contracted.

(8) Material from the lacustrine chalk of the 'Mittersee' above Lunz.

(9) Grows in putrefaction cultures with starch or starch-containing substances, covered with soil.

##### 5. *Astasia Dangeardii* Lemm. var. *parva* n.var. (Fig. 5)

(1) Shape very variable, also when swimming. Undisturbed cells are mostly spindle-shaped, during quick movement like an elongate carrot with only slight curvature, often nearly rod-shaped. Mostly narrowed and with a short prolongation towards the back



end, nearly cylindrical with a straight truncation towards the anterior end. Posterior end usually rather acute, an important feature. The body can become somewhat flattened or ventricose, but the anterior end remains drawn out like a neck with the small flagellar funnel in the middle.

Lively metabolic changes of shape as a result of the least disturbance, but there are no evident torsions.

(2) Length 30–35  $\mu$ , breadth 7–10  $\mu$  (the type 30–58  $\mu$  and 12–20  $\mu$  according to Lemmermann).

(3) Striae distinct but often delicate, when they are clearly visible only at the margin and cannot be traced over the entire surface.

(4) The flagellum is shorter than the body and is easily shed.

(5) Nucleus behind the middle or even near the posterior end, both it and the caryosome very transparent so that they are difficult to distinguish in the living individual.

(6) Paramylon grains small, spherical to oblong or shortly rod-shaped.

(7) Locomotion often erratic, vibratory. The anterior end is bent a little to one side and traverses a spiral course. Conglomerations formed in drops from crowded cultures with soil and starch.

(8) There is a certain similarity to *A. Klebsii*, from which *A. Dangeardii* was first separated by Lemmermann. The differences consist mainly in the more distinct striation and in the lack of torsion in *A. Dangeardii*. This was pointed out by Lemmermann, and I have therefore referred my strain to *A. Dangeardii*, since its shape and surface markings are also to some extent comparable, although the dimensions of my strain are less.<sup>1</sup> A change of name may become necessary if the true *A. Dangeardii* is rediscovered.

The strain also shows a certain resemblance to my *A. granulata*, which is, however, usually broadest near the anterior end, is liable to strong torsions and possesses the short posterior prolongation; and to *A. gomphonema*, which, however, is considerably smaller and never possesses a pointed posterior end.

(9) Material from the neighbourhood of São Paulo in Brazil from water with rust-red mud.

(10) Multiplies well in putrefaction cultures with substances rich in starch and covered by soil.

#### 6. *Astasia fustis* n.sp. (Fig. 6)

(1) In the free-swimming condition the body is approximately club-shaped (hence the specific name), the thicker end being that which bears the flagellum, while the posterior end is gradually narrowed or cylindrical, but not acute or prolonged. The anterior part may possess nearly parallel edges up to the foremost extremity which is somewhat narrowed. Front end broadly truncate, but not with an elongate neck as in the other species of the *A. curvata* group, being often thicker there than immediately behind.

There is no funnel at the base of the flagellum, but a narrow channel. The gullet could not be detected among the numerous paramylon grains, even after fixation and staining.

While swimming freely the cell rarely undergoes metabolic changes of shape, although sometimes it is somewhat curved. Under a cover-glass there is no free swimming, but lively metabolic movements are observed, with strong torsions and flattening as well as

<sup>1</sup> The great range of size given by Lemmermann suggests that his material of *A. Dangeardii* was not homogeneous.

spherical metaboly, similar to that of *Distigma proteus*. The contorted shapes often appear rather regular, but while creeping the cell usually becomes more irregular.

(2) Length 70–85  $\mu$ , breadth 9–12  $\mu$ .

(3) Striation often very distinct, but sometimes delicate and only visible at the margin of the optical cross-section.

(4) Flagellum shorter than the cell, about three-quarters of its length. Sometimes straight, so that it can be measured: 56–60  $\mu$ .

(5) Nucleus subcentral, mostly somewhat behind the centre, no caryosome visible in the living condition.

(6) Paramylon grains, as usual, broadly elliptical and compressed, sometimes in part elongate, rarely even nearly needle-shaped, most situated in the anterior part. During metabolic contraction the grains assume a different position according to their situation

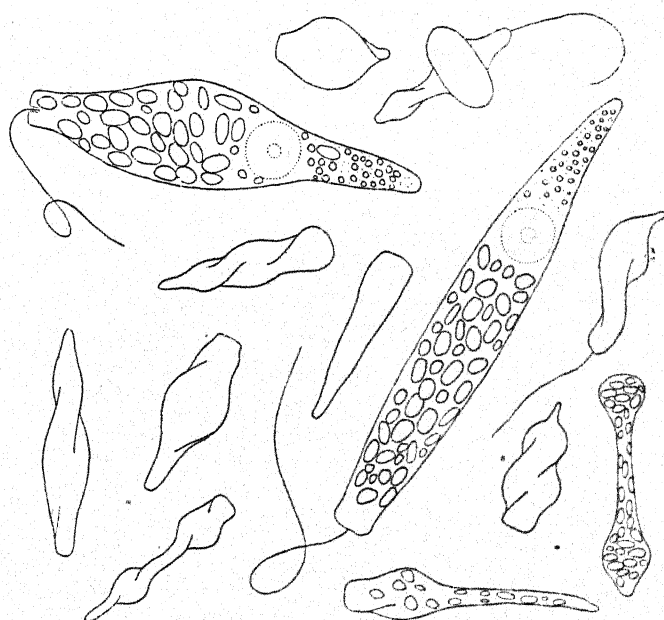


Fig. 6. *Astasia fustis* n.sp. This is the largest metabolic *Astasia* hitherto found; the larger figures show the shapes seen in free-swimming individuals, the smaller ones the metabolic changes resulting from disturbances.

in the cell. In elongate cylindrical parts of the body the long axis of the paramylon grains is parallel to that of the body, in rounded ones at right angles to it (cf. Fig. 6, individual in the right lower corner).

In addition to the oval paramylon grains small exactly spherical granules are often found, similar to those in the cells of *A. granulata* and of *Menoidium*. They occupy the posterior part of the body.

(7) The swimming movement, as long as undisturbed, is steady, although not very rapid, and sometimes spiral owing to a slight bending of the body.

(8) Similar to *A. fustis* is *A. granulata* which, however, is half the size and possesses a posterior process which is mostly not found in *A. fustis*. Further, it is not as elongate as the latter when swimming freely. *A. Klebsii* Lemmerm. is very similar, but differs in its

torsions which rarely cease even during swimming and in its mostly narrowed, not broadened, front end; my strain (cp. 1936, p. 65) also in its smaller dimensions; according to the original diagnosis by Lemmermann its striation should be very faint, which is often well marked in *A. fustis*. Korshikov's (1928) *A. Skadovskii*, the length of which is about  $70\mu$ , is similar to *A. fustis* in shape and behaviour, but its front end 'terminates with a large and widely open funnel with the mouth attaining  $5-6\mu$  in width'.

(9) Obtained first from mud with a variety of interesting flagellates, sent to me by Prof. T. M. Harris, University of Reading. In the enrichment cultures it occurred together with *Rhabdomonas costata* and *Astasia Harrisii*.

(10) Putrefaction cultures with starch-containing substances, soil and chalk.

Other strains were found in a garden pool in Cambridge and in a ditch at Cherry Hinton near Cambridge.

### 7. *Astasia gomphonema* n.sp. (Fig. 7)

(1) The most characteristic shape is like that of certain species of the diatom genus *Gomphonema*, i.e. there is a large nearly spherical 'head' and a smaller pear- to club-shaped 'body' with an intermediate constriction. There is often also resemblance to the body of an ant. The cell can sometimes be more elongate, the major part becoming nearly cylindrical with a slight conical narrowing towards the back end. The anterior end, however, always remains capitate, while the posterior end is never pointed. Cells mostly straight, rarely assuming the form of a slightly curved club. When strongly contracted a median enlargement may develop which may even become larger than the 'head', so that the ant-like shape is attained which is so striking in freshly mounted preparations, which have not yet assumed their normal condition. Finally, strongly metabolic cells may become nearly violin-shaped, with a slight torsion, or even flattened with a lemon-like outline. This marks the highest degree of contraction.

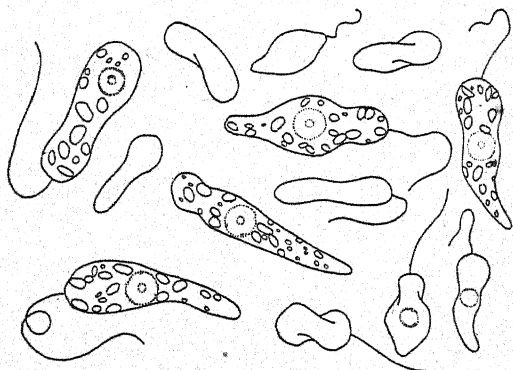


Fig. 7. *Astasia gomphonema* n.sp. All the cells were more or less twisted, but this could not be depicted satisfactorily in outline drawings.

- (2) Length  $22-25\mu$ .
- (3) Striation oblique and very delicate, but distinct and with relatively wide interspaces between the striae.
- (4) Flagellum of about the same length as the uncontracted body, easily shed.
- (5) Nucleus in the central or posterior part of the body, transparent; the caryosome becomes visible after addition of iodine.
- (6) Paramylon grains short cylinders, often angular, all of about the same size, except towards the posterior end, where they tend to become scantier and smaller.
- (7) Manner of swimming not characteristic.
- (8) Differences from *A. inflata* are the more delicate striae, the longer flagellum and the absence of large stratified paramylon grains; from *A. clava* the absence of a true club-like shape, not drawn out into a long posterior process.

(9) From the 'Obersee' and 'Untersee' near Lunz in Lower Austria, living in rather pure water, not in mud.

(10) Putrefaction cultures with starch-containing substances and soil.

# 8. *Astasia granulata* n.sp. (Fig. 8)

(1) Among the small species this is the one that exhibits most change of shape. During all metabolic variations, however, a nipple-shaped posterior stump nearly always persists and constitutes the most characteristic feature of the species. Only when the highest degree of stretching is realized, does the narrow back end gradually merge into the main body, but mostly it is distinctly demarcated and persists as a special tail even after fixation.

A characteristic shape is lageniform, the broader and narrower ellipsoidal portions being separated by a shallow median waist. The somewhat truncate anterior end can then be compared to the bottom of a wine bottle, while the 'tail' is equivalent to the neck. When the cell is most strongly stretched, it may assume a long club-shaped form, the flattening at the flagellar pole and the small point at the opposite end, mostly remaining preserved. Nearly cylindrical to carrot-like shapes are also encountered. When strongly contracted the middle part of the cell is rounded, while the ends are shortly cylindrical, the whole body resembling a Chinese lantern, though in general somewhat twisted. Torsions are altogether to be seen in all metabolic forms which are not fully stretched. After strong disturbance the flagellum is shed and spherical metaboly, accompanied by protoplasmic streaming, is seen. The anterior end, which nearly always remains broad, and the posterior end with its blunt process, mostly lie in one straight line; the body as a whole is therefore not curved.

After fixation in formalin, corrosive sublimate or osmium tetroxide, the shrunken cytoplasm usually separates from the stiffened periplast, a feature not seen in other species.

(2) Length when extended 30-40  $\mu$ , breadth 8-12  $\mu$ .

(3) Striation not very dense but distinct, for the most part readily recognized even with medium power magnification.

(4) Flagellum up to the length of the body.

(5) Nucleus generally in the middle or somewhat behind, recognizable as an area free from paramylon grains. Caryosome visible only after fixation.

(6) Paramylon grains for the most part small and ovoid, some also flattened and lying opposed by their flat faces. These grains usually fill the cell from the anterior end to the neighbourhood of the nucleus. Some of them may be distinctly longer than the rest.

In addition, innumerable exactly spherical small granules, all of almost the same size,

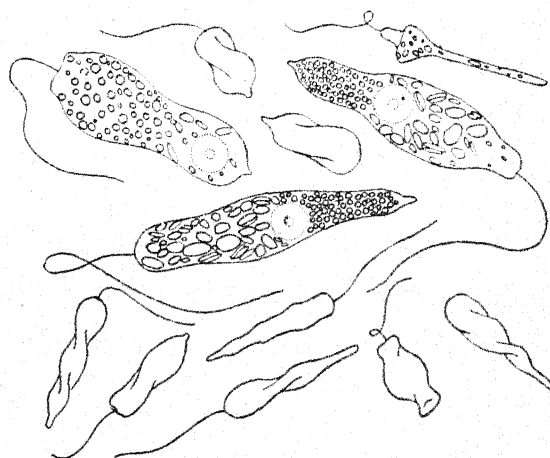


Fig. 8. *Astasia granulata* n.sp. The small spherical granules, to which the specific name is due, are accumulated in a characteristic manner in the back end in nearly every cell as long as the cultures are not too old.



occupy the posterior part of well-fed cells. Hence the specific name. I am not certain that they are of the nature of paramylon, although they present the same appearance and, like the latter, are not coloured by iodine. In rare instances, probably in starving cells, they are lacking or, on the other hand, fill the entire cell. In such a case they replace the larger paramylon grains, or again, in the posterior end their place is taken by a fine grey mass which cannot be resolved with the microscope. These small spheres often extend to the region where the structures, which are readily recognized as paramylon, begin, but in rare cases they may also occur intermingled with them. They strongly resemble the small spheres found in the species of *Menoidium* (Pringsheim, 1936, pp. 58, 70 et seq.).

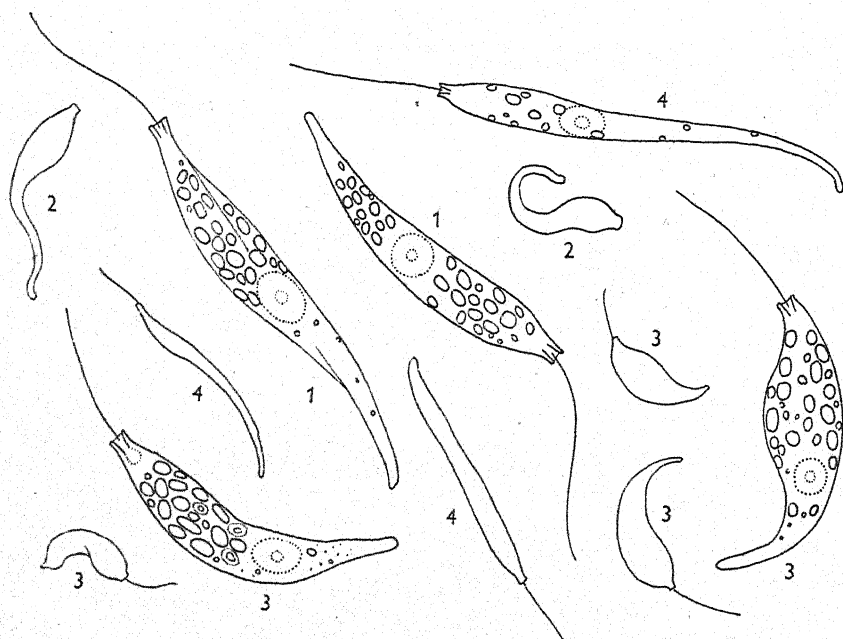


Fig. 9. *Astasia Harrisii* n.sp. The larger figures show the greatest range of shape in the free-swimming state, the smaller ones (2) the creeping movements exhibited after some time under a cover-glass. Numbers correspond to 'formae'.

(7) The manner of swimming shows no peculiar features. Conglomerations may occur in uncovered drops derived from densely populated putrefaction cultures.

(8) There is a certain similarity with my strain of *A. Dangeardii* (see under that species) and with *A. Klebsii*, which, however, is less distinctly striated and has a relatively shorter flagellum.

(9) Has been raised in cultures of material from the following localities: (1) Epiphytic growth on *Typha* in the 'Grossteich' (large, lake-like pool) near Hirschberg in northern Bohemia. (2) Semi-permanent rain pool on a shadowed forest path near Prague. (3) Highland pool above Lunz am See in Lower Austria. (4) Garden pool, Cambridge.

(10) Thrives well in a diversity of putrefaction cultures, both with starch-containing and with protein material, such as cheese, etc.

#### 9. *Astasia Harrisii* n.sp. (Fig. 9)

(1) Cells, when swimming undisturbed, slender and faintly curved, slightly flattened; mostly there is only one weak curvature, but sometimes there are two, the body being

actually spiral. Front end similar to that of *A. curvata* and *A. torta*. The broadest part of the body is in front of the middle, while the posterior part gradually narrows, the back end being nearly cylindrical.

While swimming freely there is usually not much change of shape, and the cells often appear almost rigid. The metaboly then consists only in alterations in thickness and curvature, but when the cells cease their forward movements, even without shedding their flagella, the posterior end may undergo striking sinuous changes of shape, while the outline of the anterior end remains nearly as before. This metaboly of the posterior part is accompanied by lively protoplasmic streaming throughout the whole cell, in the course of which the paramylon grains are likewise moved to and fro. No distinct torsions and no spherical metaboly.

Behind the slightly protruded and funnel-like front end, the gullet is sometimes visible. It is remarkably small.

The shape of this species depends much on the surrounding conditions. These influences cannot be satisfactorily defined yet, owing to the fact that *A. Harrisii* could not be grown in pure culture.

We can, if we like, discern four 'formae':

(1) f. *taeniformis*, a modification with a flattened body, similar to that of *A. curvata* Klebs. It occurs often in cultures with starch and soil.

(2) f. *serpens*, found in very old cultures with starch and soil, and characterized by its metabolic back end.

(3) f. *lunula*, typical for cultures with cheese. This modification is considerably shorter and thicker than the others.

(4) f. *elongata*, as found in cultures with one wheat grain and soil. Body very slender, faintly and irregularly curved.

For all these cp. Fig. 9 and its explanation.

(2) Length of the stretched body 45–65  $\mu$ , breadth about 5–6  $\mu$ .

(3) Delicate striation may be visible above the nucleus and the gullet, where the paramylon grains do not interfere with transparency as much as in other regions of the body, but scarcely at the margin of the cell as in other species.

(4) Flagellum about one-third of the length of the body.

(5) Nucleus central or a little behind the centre, somewhat compressed. In the living cell the caryosome is not visible.

(6) Paramylon grains usually only in the anterior part, often extending to the nucleus, sometimes even to the back end, elliptical, somewhat flattened.

(7) The cell in movement pursues a spiral course owing to the curvature of the body which rotates on its long axis while swimming. No creeping.

(8) There is a marked resemblance to *A. curvata* in the shape of the front end and the curvature, but the kind of metaboly is different, and the new species is larger than my strain of *A. curvata*. According to the figures reproduced by Lemmermann (1913), '*Menoidium tortuosum*' Stokes (cf. p. 32) seems to be similar in size and shape to *A. Harrisii*, but it is stated to be rigid. If free-swimming individuals only were examined and the metaboly had escaped notice, our new form might erroneously be referred to *Menoidium*.

(9) Derived from the same enrichment cultures as *A. fustis* from Reading and from a garden pool in Cambridge.

(10) Putrefaction cultures with starch and soil and a small quantity of chalk.

10. *Astasia parva* n.sp. (Fig. 10)

(1) Club-shaped and slightly curved while swimming freely, rounded at the anterior, gradually narrowing to the posterior end, but not pointed and without a pronounced process. While swimming, the posterior end may also become somewhat thickened so that the cell comes to be nearly cylindrical. Lastly, the posterior end can also undergo a button-like thickening. Torsions occur but no median constriction. The insertion of the flagellum is slightly lateral.

(2) Length of the body after fixation in Flemming's solution, when elongated, 22–25  $\mu$ , breadth 5–6  $\mu$ .

(3) Oblique striation, very delicate.

(4) Flagellum about as long as the uncontracted body.

(5) Nucleus about in the middle of the cell, difficult to recognize during life and like the caryosome clearly visible only after staining.

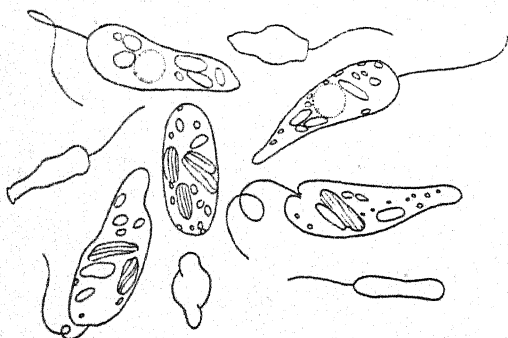


Fig. 10. *Astasia parva* n.sp. Nucleus not readily detected. The peculiar structure of the paramylon grains is obvious, despite their small size.

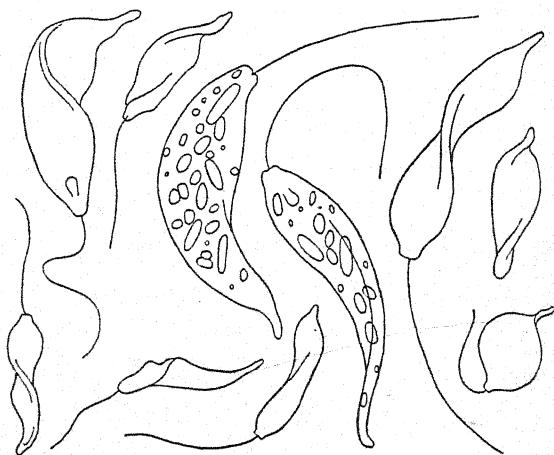


Fig. 11. *Astasia torta* n.sp. The extraordinary diversity of shape and the special configuration cannot be clearly shown by a limited number of figures, while the constantly changing form cannot be depicted at all.

(6) Paramylon grains elongate to rod-shaped, without stratification but often showing longitudinal striation, the cause of which is not clear.

(7) Pursues a rather straight course when swimming, otherwise no special features.

(8) Shape similar to that of *A. comma*, but the cells are smaller and less distinctly striated. The nucleus lies nearer to the anterior end.

(9) From an alder-marsh by the 'Untersee', Lunz.

(10) Thrives well in putrefaction cultures with starch-containing material covered with soil or peat.

11. *Astasia torta* n.sp. (Fig. 11)

(1) Cell when swimming undisturbed elongated and slender, widest near the anterior end, gradually thinning towards the posterior end, generally somewhat spirally twisted with a steep spiral. Back end finely drawn out and sharply acute. Anterior end slightly protruded and narrowly truncate. Cells often also approximately club-shaped, the

anterior end then being somewhat flattened and twisted. At times the surface of the body is irregular with rounded protrusions which are not caused by paramylon grains.

In microscopical preparations the cells mostly appear almost flattened and ribbon-like and twisted as a whole through  $180^{\circ}$ . As a result of strong disturbance the body then becomes curved, so that the anterior and posterior ends do not lie in the same straight line but are inclined to one another at an angle of about  $100-120^{\circ}$ . Even then, however, not more than half a torsion is evident. The short almost cylindrical tail does not disappear even during the strongest metaboly.

During fixation the body may become stretched so that the torsion disappears and an almost rod-like shape is assumed, such as does not occur in living cells. Spherical metaboly, protrusion of thin parts of the body and creeping movements on a solid substratum have not been observed.

(2) When fully extended the cells have a length of  $30-38\mu$  and a breadth of  $7-8\mu$ .

(3) Striation, usually distinct, oblique, not of the same kind in all cells, sometimes very delicate.

(4) The flagellum is robust. Its length can be estimated, since it becomes rigid and straight in a solution of methylene blue like the flagella of *Menoidium* or *Rhabdomonas*; it is about that of the body or slightly less.

(5) The nucleus is about central.

(6) Paramylon grains small, rounded rod-shaped, in part 3-4 times as long as broad. The posterior part of the cell usually contains only minute granules in small numbers, so that this part of the cell appears almost empty.

(7) The manner of swimming is not very characteristic. It is rather slow, especially in cells that are appreciably curved. No creeping.

(8) There is a certain similarity between this species on the one hand and *A. Klebsii* and *A. curvata* on the other, the new species occupying almost a middle position between the two. It is not as long and not as markedly twisted as *A. curvata* and does not curve to the same degree. From *A. Klebsii* it differs in being markedly ribbon-shaped, in its curvature and in the lesser breadth of the anterior end. Furthermore, *A. torta* is smaller than the two other species.

(9) It was reared for the first time from material from a highland pool at the Plöckenstein above Lunz, which on immediate investigation displayed only *Trachelomonas*. Two other strains were isolated from ditches of the Kummerteich meadow near Hirschberg in Bohemia, where *Trachelomonas*, accompanied by Desmids, was likewise observed. A fourth strain was obtained from material growing on stems of *Typha* in the Grossteich near Hirschberg.

(10) Multiplies well and quickly in all kinds of putrefaction cultures with starch-containing substances.

## 12. *Astasia linealis* mihi (Fig. 12)

No figures of this species were published in the earlier communication (1936, p. 78), since that of Klebs (1883, Tab. II, fig. 10) seemed to be satisfactory. But as several similar forms have been detected, I am now giving drawings of my strain showing also the vacuolar system which is particularly well seen in this species.

The inner part of the large gullet is nearly spherical, and the reservoir is immediately behind it. The two are separated by a plasma-lamella which is plane as a result of the mutual pressure. After some time this lamella suddenly breaks, and the rounded posterior margin of the reservoir



shifts rapidly forwards until it vanishes, while the liquid contained in it is poured into the gullet. Immediately afterwards a new reservoir is constituted which at first is very small, with an ill-defined margin. No accessory pulsating vacuoles could be detected.

In *Euglena acus* the vacuolar system is altogether similar except for one feature. The reservoir here lies not directly behind the gullet, but to one side, and the intervening plasma-lamella is much smaller. The difference may be due to the fact that the gullet is respectively central and oblique in the two species.

From a reference of Skuja's (1939, pp. 111-12) I gathered that Korshikov (1917) has described a *Cyclidiopsis acus* which may possibly be identical with *Astasia linealis*. Skuja found this species again, but both his and my forms had an elongate nucleus unlike the spherical one drawn by Korshikov. It is probable that his observation is erroneous.

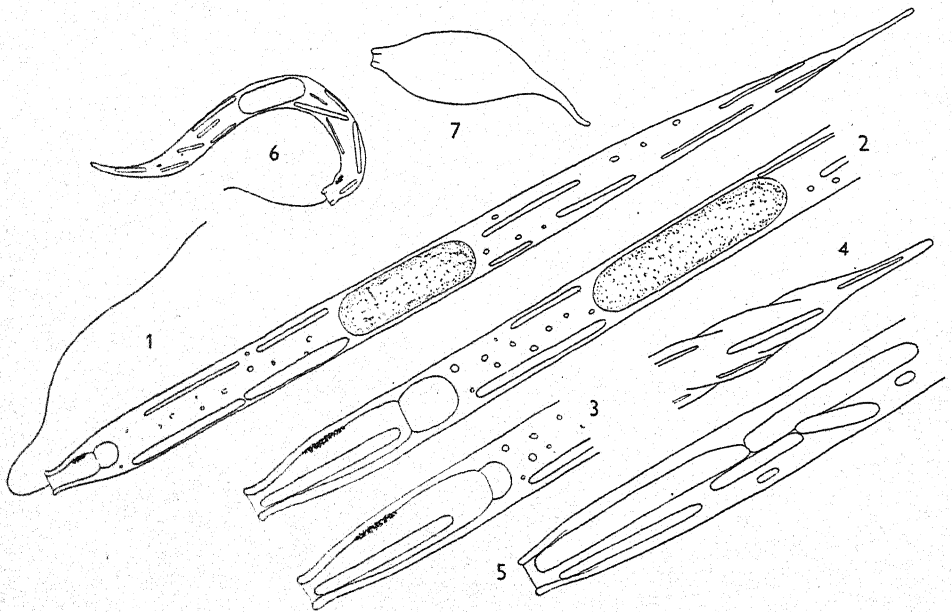


Fig. 12. *Astasia linealis mihi*. (1) Normal individual with few paramylon grains, from a healthy culture ( $\times 1000$ ). (2), (3) Front ends of two individuals from the same culture, somewhat more magnified to show the mechanism of the contractile vacuole. (2) Shortly before the liquid is discharged from the reservoir into the gullet, (3) soon after discharge when a new reservoir is forming. (4) Posterior end of a cell, on the same scale. (5) Front end of an individual with plentiful paramylon. (6) Strongly curved individual ( $\times 500$ ). (7) Injured and swollen, but still living individual, from the sediment of an old culture ( $\times 500$ ); such cells may again become healthy under favourable conditions and resume division.

Skuja cites four characters in which *Cyclidiopsis acus* is claimed to be different from *Euglena acus hyalina*, an apochlorotic variety first described by Klebs and found again by Skuja. I did not see it and have only been able to compare two green strains of *Euglena acus* with my *Astasia linealis*. (1) According to Skuja the chief characteristic of *Cyclidiopsis acus* is the central gullet, while *Euglena acus* and its hyaline variety have a one-sided one. But the degree of eccentricity varies greatly among the forms belonging to the group or collective species *Euglena acus*. *Cyclidiopsis acus* corresponds only to one extreme. The strains of *Euglena acus* I could compare possess a nearly central gullet, not located as much to one side as in the figures given by Skuja (Tab. VI, fig. 6-10). (2) The sausage-shaped nucleus of *Cyclidiopsis acus* is a valuable characteristic, but the difference in this respect between *C. acus* and *Euglena acus* is in no way so considerable as Skuja supposes.

The nucleus of my strain of *E. acus* is in life likewise a cylinder with two rounded ends, though it is not so elongate as that of *Astasia linealis*. (3) A comparable difference is found in the paramylon grains. Skuja speaks of them as 'quite different in form', but in every strain of the *Euglena acus* group they are rod-shaped and more or less thin and pointed according to the quantity of paramylon that has been stored. Those of *Astasia linealis* are more needle-shaped, it is true, but the difference is again only one of degree. (4) *Cyclidiopsis acus* is described by Skuja as completely rigid, while *Euglena acus hyalina* shows a slight degree of metaboly. My strain of *Astasia linealis* is liable to exhibit curvature like my *Euglena acus*.

In order to explain why I do not agree to the separation of *Cyclidiopsis* as a distinct genus I will enumerate the features which are common to *Astasia linealis* and the other members of the *Euglena acus* group: (1) The highly elongate shape which in *E. acus longissima*<sup>1</sup> is as slender as in *Astasia linealis*. (2) The posterior spinous process which under certain circumstances adheres to the substratum by its tip. (3) The presence and the shape of the stigma, although it is fainter in *A. linealis* than in *Euglena acus*. (4) The type of metaboly with curvature of the body, while the spine remains straight and is merely bent to one side. (5) The very delicate oblique striation. (6) The more or less cylindrical shape of the nucleus. (7) The shortness of the flagellum. (8) The rod-shaped paramylon grains. (9) The vacuolar system.

For these reasons I adhere to the name *Astasia linealis* as long as the difference between *Euglena* and *Astasia* is based only on the presence or absence of chlorophyll, and I cannot accept the genus *Cyclidiopsis*. The specific name *acus* is likewise unsuitable, since my form seems to be slightly different from that described by Korshikov.

### 13. *Astasia longa* mihi (Fig. 13)

Lastly, I wish to refer again to *Astasia longa*, of which I give new figures showing the typical state exhibited by the cells when moving in a drop of culture medium without a cover-glass (Fig. 13A). Lwoff & Dusi (1934) have also isolated a clone of this species and described it under the name *A. Chattoni*.

My continued use of the name I gave to the species does not imply any lack of respect towards Lwoff or Chatton. When, however, Provasoli (1937-8, p. 77) claims that the name *A. Chattoni* has priority in accordance with the rules of nomenclature, he is mistaken. As I explained already in 1936 (p. 94), Lwoff & Dusi published no figure, and their description was insufficient to such a degree that no one would have succeeded in recognizing the species. I myself would have been unaware that it was identical with *A. longa* had not Lwoff had the kindness to send me his strain. The double name is therefore not, as Provasoli believes, due to the lack of knowledge on my part of the paper of Lwoff & Dusi, as will be apparent from my addendum (1936). Provasoli is also mistaken concerning the differences in the statements as to the dimensions of the cells. They do not depend on my strain being longer and narrower than that of Lwoff & Dusi. Both are completely identical with one another and with four strains which I have isolated since, but I thought it essential to measure fully extended individuals, because data obtained otherwise are not of much value. These remarks appeared necessary because there is a risk that the same organism may be referred to in the literature under two different names.

Having made further studies of *A. longa* I have to add certain corrections and additional details: (1) *Striation*. It has been stated that *A. longa* has a smooth periplast, but if the fixed cells are allowed to dry on the slide and are then covered with a colloidal dye, very

<sup>1</sup> This 'variety' and the 'species' *Euglena acutissima* are probably mere modifications of *E. acus*.

faint and delicate striations become visible. The best results were obtained with collargol and with Klein's silver-nitrate method (Klein, 1930). It seems probable that spiral striations are not lacking in any member of the Euglenineae. (2) The *position of the nucleus* was given as 'mostly behind the middle'. In a newly isolated strain it struck me that the gap between the paramylon grains pointed to an almost central position of the

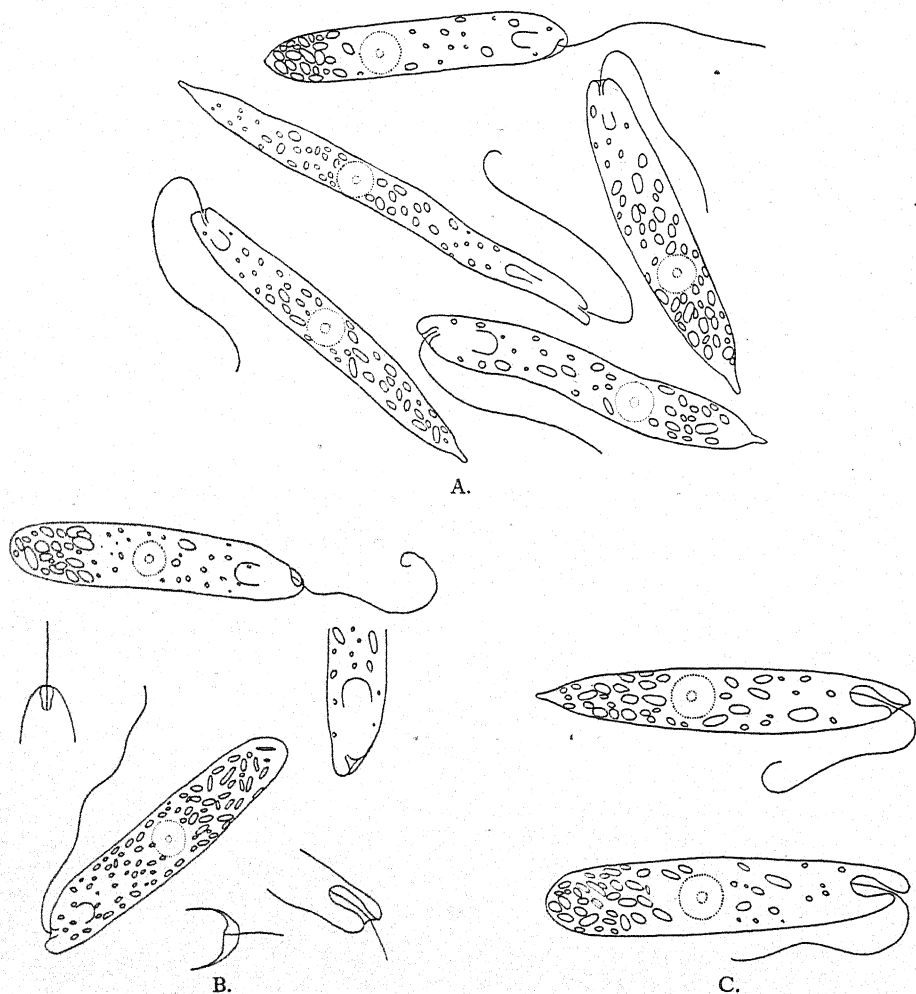


Fig. 13. *Astasia longa mihi*. A. Typical individuals of the species which nearly always show the posterior process; in an earlier communication I published drawings of not quite typical individuals which has given rise to misunderstandings. B. Var. *truncata* n.var. Cells always devoid of the pointed posterior process. Single individuals of the type may be mistaken for the variety; in order to determine that the variety is unable to form the posterior process numerous cells from many cultures had to be examined. C. Cells of the two forms for comparison (semi-diagrammatic).

nucleus. Renewed examination of several strains proved that this is often the case, so that it is better to say: nucleus central or behind the middle. (3) *Flagellum*. My earlier statement as to the length of the flagellum agrees with that of Lwoff & Dusi, taking into account that they observed cells which were not completely extended. Actually, however, it is longer, as I have found by the examination of several strains and may even reach a

length of  $35\mu$ , while the body has a length of about  $55\mu$ . I have previously described the flagellum of *A. longa* as ribbon-shaped. As Vlk (1938, p. 469 et seq.) has recently shown, all Euglenineae probably possess the ciliate flagellum already demonstrated by Fischer (1894) and Mainx (1927, p. 311). This will also be valid for *A. longa*, the border of cilia giving the impression of a ribbon. (4) *Shape of the body*. Especially characteristic is the elongate, almost cylindrical form with the oblique anterior truncation and the posterior apiculus. The latter on rare occasions is drawn in, and it seems to be lacking completely in a new strain (cf. *Euglena gracilis* var. *urophora*, Provasoli, 1937-8, p. 23, Figs. 13 and 14 and p. 74). Since this strain agrees in all other respects and also multiplies in an acetate-peptone medium, a separation from the 'main species' is scarcely possible. This form, which has proved to be constant, may be described as *A. longa* var. *truncata* (Fig. 13 B, C). It is derived from a sample collected in the neighbourhood of Hirschberg in Bohemia.

### (3) Table for identification of species

An identification key, such as is usually most suitable for practical purposes, is not feasible here, because the species of *Astasia* cannot be distinguished without taking into consideration several characters. The grouping adopted in the following scheme cannot be regarded as more than an imperfect attempt.

**Group 1.** Cells elongate and slender, rather large, liable to strong torsions. Anterior end protruded and transversally truncate. There usually is a short posterior process.

A. Metabolic shape of the body ribbon-like. Flagellum shorter than the body.

B. Body twisted during metaboly but not flat. Flagellum about as long as the body.

- A. *a.* About  $40-45\mu$ . The shape, which has given the species its name, is that generally seen in a microscopical preparation. Cells appear narrow, ribbon-shaped, twisted several times. When strongly contracted the cell as a whole is curved in an approximately semi-circular manner. Under a cover-glass this shape is kept for a long time and the 'normal' form is never seen. In an open drop, however, the cells straighten after a while and then do not betray any curvature or twisting. Striation distinct, though delicate. Flagellum two-thirds of the length of the body. Anterior end broadly protruded. No spherical metaboly.

**A. curvata** Klebs.

- b.* About  $45-65\mu$ . Similar to *A. curvata*, but usually less ribbon-shaped and twisted, only slightly curved to one side, sometimes in two opposite directions. When swimming undisturbed almost straight. Striation hard to recognize. Flagellum one-third the length of the body. Anterior end protruded like a broad neck. Posterior end may exhibit snake-like movements. No spherical metaboly.

**A. Harrisii** n.sp.

- c.* About  $30-38\mu$  long, less twisted than *a*, torsion through no more than  $180^\circ$ . If swimming freely, slender and straight. Not bent more than in an obtuse angle, even when strongly contracted. Striation faint, not visible in all cells of a population. Flagellum nearly as long as the body. Anterior end little protruded, appearing as a narrow truncated neck. No spherical metaboly.

**A. torta** n.sp.

- B. *d.* About  $40-50\mu$ , rarely distinctly ribbon-shaped, often twisted several times, not like a cork-screw but round the longitudinal axis of the body. If the cell swims undisturbed the spiral twisting gradually disappears and eventually the body becomes straight and club-shaped. Striation hard to recognize. Flagellum about as long as the body. Anterior end broadly protruded and truncate. Spherical metaboly and creeping movements frequent.

**A. Klebsii** Lemm.

- e.* About  $70-85\mu$ . Similar to *A. Klebsii*. Never ribbon-shaped. Torsions similar to those of '*d*', but less marked. When swimming undisturbed resembling a club with a thick end. Metaboly with pronounced torsions and creeping movements with spherical con-



tractions occur. Striation more distinct than in 'd'. Flagellum about three-quarters the length of the body. Anterior end always broadly truncate.

**A. fustis** n.sp.

- f. About 30–40  $\mu$ . This species does not quite conform to the diagnosis of the group but it reveals a relation to *A. Klebsii*. Cell never ribbon-shaped. Torsions during metaboly very strong. During free swimming they disappear after some time, and the body becomes club-shaped but the truncation of the anterior, and the nib-like process at the posterior end mostly remain distinct.

**A. granulata** n.sp.

*A. granulata*, *A. Klebsii* and *A. fustis* can be arranged in a series of growing size and appear to be somewhat related. All these three species contain pearl-like granules in the posterior part of the body. There is no strict proportionality between the size of the body and that of the flagellum, so that the relative length of the latter is least in the largest and highest in the smallest species.

**Group 2.** This group contains numerous small species which are difficult to distinguish. Common to all of them is the pronounced metaboly, with little tendency towards flattening or torsion. While swimming freely the cells generally appear comma-shaped, exhibiting a slight curvature of the club-shaped body.

- a. Length 20–25  $\mu$ , broadly club-shaped, rarely slender and almost straight, but mostly curved. This species shows the greatest tendency to flattening and torsion among the group. In the anterior part a demarcated 'head' can appear, similar to that of species 'd'. Anterior end oblique. Flagellum one and a half times as long as the body. Striation very delicate.

**A. clava.**

- b. Length 28–32  $\mu$ . More slender than the preceding species, generally curved, front end not oblique. Flagellum as long as the cell, striation distinct.

**A. comma.**

- c. Length 30–35  $\mu$ . Broadest part of the body approximately in the middle, front end not oblique. Flagellum shorter than the cell. Striation distinct.

**A. Dangeardii** var. **minor.**

- d. Length 22–25  $\mu$ . Club-shaped, straight, usually somewhat truncate in front. Flattening and torsion at the most faintly indicated. Flagellum about as long as the body. Striation distinct.

**A. parva.**

- e. Length 16–20  $\mu$ . Anterior end relatively very broad, usually transversally truncate. Curvature rather considerable. By thickening of the back end the body may become cylindrical. Striation distinct.

**A. cylindrica.**

- f. Length 22–25  $\mu$ . Front end rounded, capitate and demarcated from the body. The latter often with a second inflation in the middle. Cell generally not curved. Flagellum at the most as long as the body. Striation distinct.

**A. gomphonema.**

**Group 3.** This group is rather closely related to the preceding one. The characteristic means of distinction lies in the broad, flattened shape, which has provided the specific names of the two forms included here and is assumed already as a result of slight mechanical disturbance. It is therefore regularly observed in cover-glass preparations. Other types of metaboly only appear after greater chemical stimulation.

- a. Length 40–46  $\mu$ . Diagnosis after Skuja (1939, p. 105). While swimming freely elongate pyriform, not flattened, slightly curved. Metaboly stages ovoid, slightly protruded and truncate at the anterior end, narrowed but rounded off at the posterior end. In the middle of the cell two to three relatively large rod-shaped paramylon grains.

**A. inflata** Klebs, typ.

- b. Length about 20  $\mu$ . While swimming freely obliquely club-shaped, movement rapid. When most strongly contracted the body is quite flat and the outline becomes lemon-shaped. Paramylon grains in part relatively large but short rods. The description and drawings of Skuja make it clear that these two varieties, which differ in size, are otherwise very similar.

**A. inflata** v. **minor.**

- c. Length up to 40  $\mu$ . Generally broadened at the anterior end in a spoon-shaped manner, never rounded in cross-section, usually not curved. Paramylon grains small.

**A. applanata.**

Further groups cannot at present be established. *Astasia longa* with a cylindrical body and an obliquely lateral origin of flagellum; and *A. quartana* with a lip-like protrusion and a stigma for the time being stand isolated among Astasiaceae but betray some relationship with *Euglena gracilis*. Nothing can still be said of certain species described by Playfair, Skuja and others.

Parallel to *Astasia linealis* there is, according to Klebs (1883, pp. 291 and 309), a variety or species of the *Euglena acus* group without chlorophyll which possesses an eye-spot and one devoid of it. Forms with eye-spots have been described and figured by Korshikov (1917), Playfair (1921, p. 136, Tab. VII, fig. 3), Dangeard (1930) and Skuja (1939, p. 101, Tab. VI, fig. 10 and p. 111, Tab. VII, figs. 11, 12). Since the green parallel species *Euglena acus*, appears to be an aggregate with many different subspecies, other apochlorotic forms belonging to this group may probably be found.

#### V. THE GENUS *DISTIGMA*

The type of the genus *Distigma* was established by Ehrenberg (1838, p. 116) for *D. proteus*, which was until recently the only known species. Since it was not described quite correctly, the whole genus requires revision.

According to the diagnoses and illustrations (Senn, 1900, p. 117, fig. 128 B; Lemmermann, 1910, p. 540, fig. 12), *Distigma* would differ from *Astasia* only in possessing a stump-like flagellum besides the long locomotor flagellum. After finding three new species in addition to the one recently discovered by Skuja (1939, p. 115), it is possible to give a more adequate description of the genus.

The species show the following common features: in four of them the cytoplasm is very fluid which is related to the almost amoeboid changes of shape seen even during swimming. One is more rigid. The very delicate periplast shows a striation which is difficult to observe, while the nucleus is readily visible. There are two flagella, the longer directed forwards and the short one bent to one side. Paramylon grains small.

As regards the relationship of the genus, its common features are too distinctive and peculiar to warrant placing it very close to *Astasia*. Not only the accessory flagellum, but also the low degree of viscosity of the cellular material are features which are never found in *Astasia*.

It is tempting to interpret the relationship in such a way that *Astasia*, with a single flagellum, is regarded as being derived from *Distigma*, with two flagella, by the loss of the shorter one. In a similar way one might be inclined to derive *Euglena* from *Eutreptia* by regarding the bifurcation of the basal part of the flagellum as a relict of the biflagellate state. But we are here confronted with the same difficulties as in the apochlorotic genera. The peculiar, very active metaboly of *Eutreptia* is not found in the same form in any *Euglena*, and it is probable that other characters of the genus *Eutreptia-Eutreptiella* will be found which likewise do not conform to those of *Euglena*. On the other hand, it seems possible that *Eutreptia* which agrees with *Distigma* in possessing two flagella, the special kind of metaboly (Klebs, 1893, p. 359) and minute paramylon grains, may resemble the ancestors of *Distigma*. To establish this, however, further research is necessary.

Just as few biflagellate green Euglenineae are known by contrast to the many uniflagellate ones, there seem to be far fewer species of *Distigma* than of *Astasia*. In spite of vigorous search only two new species can be added to those previously described (one in two size variations) and both of these were only found once.

#### 1. *Distigma gracilis* n.sp. (Fig. 14)

(1) Body, when swimming rapidly, like a long carrot, rounded at the front, slowly narrowing towards the back end which thins gradually but is not pointed. Body rather slender, hence the specific name. The cell is nearly always curved to and fro, but not regularly as in a cork-screw. The little thickened anterior end is slightly notched in the middle at the point of insertion of the flagellum. Although the periplast appears delicate,

the metaboly is not so pronounced as in the earlier described species. The posterior end may show a small button-shaped dilation, but this is not large enough to give rise to a spherical thickening of the whole cell or a major part of it.

It often happens that the posterior part of the body or even almost the entire cell becomes cylindrical, while the spiral curvature mostly persists. The most regular forms have almost the appearance of a large *Spirillum*. Stronger contractions are only undergone when there is considerable disturbance. Changes of shape may also appear during swimming, but only in the form of curvatures and limited torsions.

Fixation with preservation of the body form was not achieved. After treatment with Flemming and with corrosive sublimate the protoplasm becomes opaque and the cells assume an unnatural bent rod-shaped form, at the same time shortening considerably. Formalin and iodine afford no better fixation.

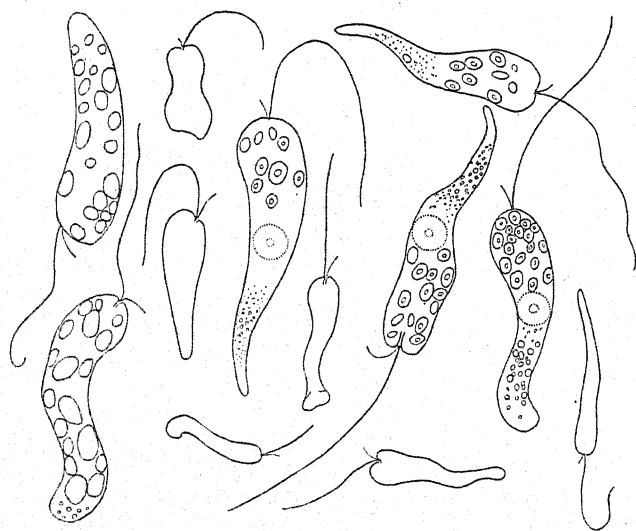


Fig. 14. *Distigma gracilis* n.sp. The slender, slightly twisted body and the *Spirillum*-like shape are characteristic of the species.

(2) Length of the body  $30-36\mu$ , breadth  $7-8\mu$ . Exact and reliable data cannot be furnished, because no measurement of the living and moving cell is possible. The cells are certainly smaller than those of *Distigma proteus* and larger than those of the two races of *D. curvata* previously described.

(3) Striation so delicate that it could not be recognized with the help of the available optical equipment.

(4) The longer flagellum is about as long as the body. The accessory flagellum is very short, about as long as half the breadth of the anterior end, i.e. about  $3-4\mu$  long. As in the other species it is slightly curved and bent to one side.

(5) Nucleus in the middle of the cell, like the caryosome distinctly visible.

(6) Paramylon grains rather characteristic, never present in large numbers, so that they are not in contact with one another. They are very minute in the narrow posterior part. Even the largest ones in the anterior part are rather small, although in most cultures they exhibited differentiation into a punctiform inner and an outer part. Other details, such as stratifications, could not be detected. In outline they are approximately ovoid.

(7) When drops were removed from very crowded cultures with starch and soil, conglomerates were formed. The movements within the swarm were tottering, owing to the curvatures and changes of shape. When swimming freely the motion is rather quick and constant, with rotation around the longitudinal axis. A 'trembling' movement like that of *D. proteus* (Pringsheim, 1936, p. 76) was rarely observed, despite the presence of the accessory flagellum, the behaviour of which during forward progression could not be determined.

(8) There is a certain resemblance to *D. curvata*, although this is shorter. Both species can readily assume a slightly curved horn-shaped form, but the slender undulate shape of the new species is a good distinctive character.

Among the species of *Astasia* which are familiar to me, there is none that could be confused with *Distigma gracilis*.

(9) Mud from the harbour of the Biological Station, Lunzer See.

(10) Starch-containing material covered with soil provides very dense cultures.

## 2. *Distigma Sennii* n.sp. (Fig. 15)

(1) Body about club-shaped, posterior end narrowing to form a handle-like cylindrical back end. Broadest part of the cell in the middle between front and hind end. Front rounded. Opening of gullet median, slightly protruded and funnel-like. Metaboly not very conspicuous so that the body often appears to be nearly rigid.

(2) Length of the body 45-50  $\mu$ .

(3) Striation not visible without special staining.

(4) Main flagellum shorter than the body. Accessory flagellum about one-tenth of the length of the longer one, but acting in the same way, slightly or distinctly curved to one side. Both appear to be of equal thickness.

(5) Nucleus central, caryosome not recognizable without staining.

(6) Paramylon grains small, ovoid, somewhat flattened, often found in the anterior part of the body only.

(7) Movement like that of an *Astasia*, not palpitating as in *Distigma proteus*.

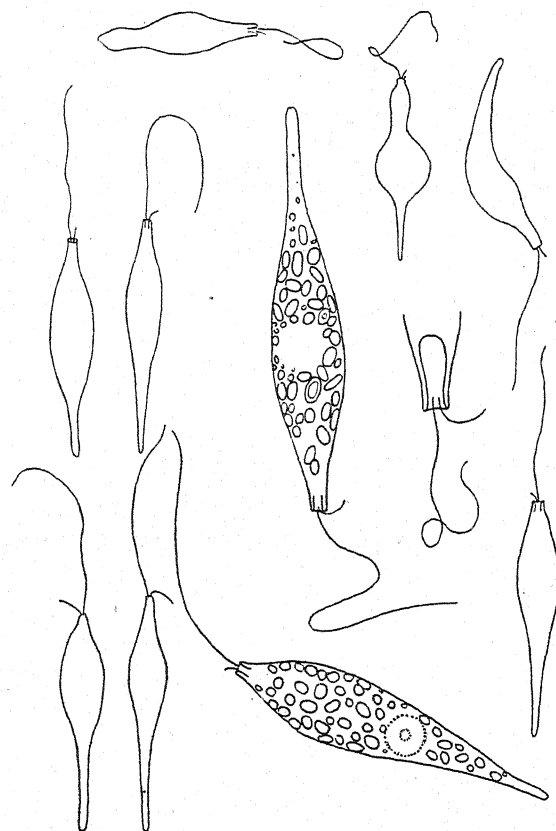


Fig. 15. *Distigma Sennii* n.sp. Two large figures show specimens with usual shape of body and grains of paramylon. Another drawing represents front end with funnel, gullet and flagella 2000 times. Metaboly is far less pronounced in this species than in *D. proteus* Ehrbg. Four small figures round the right upper corner show the greatest diversity encountered in metabolic individuals. For comparison four figures are put together at the left side, showing differences between *D. Sennii* and *D. proteus*. They are found in opening of gullet, length of small flagellum, shape of body and, not shown in the figures, much more pronounced metaboly in *D. proteus*.



(8) This species is readily differentiated from every other *Distigma* and not very similar to any *Astasia* I know.

(9) Derived from deposit of decaying organic material at the bottom of a garden pool at Cambridge.

(10) Multiplies well in every kind of putrefaction culture, with starch as well as with wheat grains, cheese, etc., just like *D. proteus*. Very crowded cultures originate in a few week's time.

### Synopsis of the species of *Distigma*

Among the strains which conform in size and shape to the type species, *Distigma proteus* Ehrbg., I have regularly found two features diverging from the usual diagnoses (Lemmermann, 1910, p. 540; 1913, p. 158), and this induced me to create a new species: *D. pseudoproteus* (Pringsheim, 1936, p. 76). This possesses not a smooth but an obliquely striated periplast, and in place of the short 'functionless' flagellum there is a motile accessory one which is much longer than that shown in the drawings of *D. proteus* as given by Lemmermann (1910, p. 517, fig. 12).

New investigations meanwhile have shown that my form actually is congruent with the original *D. proteus* Ehrbg. (1838, p. 116, Tab. VIII, fig. IV). The confusion concerning *D. pseudoproteus* was caused by various authors, such as Senn (1900, p. 117), Lemmermann (1910, 1913) and others, referring two readily discernible species to *D. proteus*, the type of which is rather common while the other form is much rarer. This has been figured first by Senn (1900, fig. 128 B). Therefore I gave it the name *D. Sennii*. Without intentionally searching for it I certainly found more than ten strains of *D. proteus* in materials from Bohemia, Slovakia, Bavaria, Holland and England before finding *D. Sennii*. It occurred in an enrichment culture derived from material from a garden pool which contained some green Euglenaceae, such as *Euglena acus*, *E. oxyuris* and *E. viridis* and a rich planktonic flora, the most abundant species of which was *Selenastrum Ehrenbergianum*.

*Distigma Sennii* is of about the same size as *D. proteus*, i.e. a length of 50–60  $\mu$ . The differences between the two species are:

#### *D. proteus*

1. Metaboly very strong.
2. Longer flagellum almost the length of the body.
3. Shorter flagellum about one-third of the longer one.
4. Broadest part of the body mostly near the front end.
5. The flagellar opening at the anterior end is narrow, the furthestmost top rounded.
6. Striation delicate.
7. Movement jerking.

#### *D. Sennii*

Not changing shape much.  
 Shorter than the body by one-half to one-third.  
 Shorter flagellum about one-tenth of the longer one.  
 Broadest part in the middle of the whole body.  
 The front top is slightly broadened, the opening of the gullet funnel-like.  
 Striation not visible.  
 Movement uniform.

There is no doubt about the meaning of Ehrenberg's name because his figure (1838, Tab. VIII, fig. IV) evidently concerns the form described as *D. pseudoproteus* in my earlier paper. Most of the figures given by other authors conform to the same species. Senn's

figure likewise is readily recognized as the form I name after him, although he gives the accessory flagellum even shorter than it is. The shape of the body is characteristically pictured and completely conform to my strain. Lemmermann in his first diagnosis of '*D. proteus*' (1910, pp. 540-1) reproduces Senn's picture in a reduced size without mentioning its origin. In his second description (1913, p. 158) he gives the same picture, adding erroneously 'after Lemmermann'.

Since the numerous strains of *D. proteus*, which have been examined from this point of view, agree with one another in all respects, it appears reasonable to withdraw the name *D. pseudoproteus* on the assumption that the previous diagnoses were not quite correct. Results of recent investigations by Lackey (1934), Hollande (1937) and Skuja (1939, p. 115) are also in agreement with this opinion. Hollande's results altogether fit my strains, as far as can be judged without repeating his cytological investigation. His statement that the shorter flagellum is the actual organ of locomotion even surpasses my conclusion that it is by no means a functionless structure, a conclusion confirmed also by the observations of Vlk (1938, p. 47). He not only states that the shorter flagellum is not a mere stump and that it is about one-third of the length of the longer one, but points out that it even has the same structure. Like the main flagellum it is covered along one side by cilia.

In addition to the four species known to me, there is also the one established by Skuja (1939, p. 115). There are thus at present the following species of *Distigma*: (1) The large and very common *D. proteus* Ehrbg. emend. Pringsheim, length 50-55  $\mu$ . (2) *D. Sennii*, which I found only once. It is of about the same size but is almost rigid. (3) The rare, smaller *D. gracilis*, 30-36  $\mu$ . (4) The frequent *D. curvata* mihi, found in two size variations, with a length 15-18 and 18-25  $\mu$  respectively. Both varieties have been found again by Skuja (1939, p. 114). (4) *D. globifera* Skuja, 20-24  $\mu$  long.

## VI. THE GENERA *MENOIDIUM* AND *RHABDOMONAS*

### (1) General discussion

The genus *Menoidium* was established by Perty (1852) and was based on *M. pellucidum*. I have not seen this species in the form figured by the author (reproduced by Lemmermann, 1913, p. 158, fig. 331). The form, which I previously called by this name, looks somewhat different, and so do drawings published by other authors. The existence of a considerable number of similar forms is proved by my strains, as well as by those described by other authors.

Klebs (1893, p. 360) referred the *Rhabdomonas incurva* of Fresenius to *Menoidium*, although in his big memoir on Flagellata (1883, pp. 294, 323) he still called it by the original name. I do not think this was a profitable decision. Where there is a reliable and natural distinction, it is better to maintain it. Although I followed Klebs at a time when my knowledge of these forms was inadequate, I prefer to change the names of certain species before it is too late. The genus *Menoidium*, in the limits at present usually adopted, includes the strongly flattened species which are related to *M. pellucidum* and the more or less cylindrical ones which centre around *M. incurvum*. No intermediate forms are known between the two groups, and the morphological gap between them is

appreciable.<sup>1</sup> It would therefore be advantageous in the future to refer to *Menoidium* only those species which correspond to the type of *M. pellucidum*, while for the non-flattened species which present quite a different appearance the old name *Rhabdomonas* Fresenius is maintained. This procedure is also justified by the consideration that quite a number of species can already be referred to the two genera. The generic names are, moreover, suitable, for the species of *Menoidium* are actually about sickle-shaped (*Menoidium* = selene menseides = sickle-shaped moon) and those of *Rhabdomonas* more or less rod-shaped.

The general characteristics of the genus *Menoidium* are: Euglenineae with a rigid, flattened body, devoid of chlorophyll. The outline of the cell, when presenting one of its broader faces as it usually does when not swimming, is bounded on the one side by a nearly straight and on the other by a curved line. The former corresponds to an acute edge, the latter to an obtuse one, the cross-section of the cell being a triangle with an acute apex. The shape can also be likened to the slice of an orange, although it is thinner and with a sharper edge in the species with which I am familiar. The anterior end is protruded probably in all species. Viewed from the narrower face, the cell appears keel-shaped, thinning out gradually towards the posterior end. The surface shows very faint and delicate striae, the course of which requires further investigation. As a rule a few of the paramylon grains are strikingly large, being shaped like rods or elongate rings, while near the posterior end there are a large number of small and exactly spherical ones.<sup>2</sup>

By contrast the cells of *Rhabdomonas* are not flattened, but have the shape of slightly curved almost terete rods. They possess a few, coarse, spiral ridges which at the anterior and posterior ends terminate in a contorted group. The flagellar pole is not protruded. The paramylon grains do not include especially large and striking forms, but the small spherical granules are found also in species of this genus. Vlk (1938) has shown that *Rhabd. costata*, syn. *Menoidium longum*, possesses a ciliated flagellum.

Common to both genera is the absence of pigment, the lack of metaboly and the coarse flagellum, which is usually directed straight forwards and often preserves this position after death. In addition there are the small spherical granules mentioned above.

## (2) *New species*

The members of the genus *Menoidium* are uncommon, as emphasized also by Playfair (1921, p. 137), and I have so far only become acquainted with three forms, all of which have not yet been described.

### 1. *Menoidium cultellus* n.sp. (Fig. 16)

(1) Like the two following species, this one is recognized at the first glance as belonging to the group of *M. pellucidum*. It is characterized by the demarcated posterior end, which is nearly square when the individual is viewed from the broad side, and by the extreme flattening of the cell, which is almost as thin as the blade of a knife, hence the specific name (*cultellus* = small knife). Seen from the narrow side, the cell therefore appears especially slender and rather pointed at the posterior end. The square terminal part is not

<sup>1</sup> The figures of *M. gibbum* given by Skuja (1939, Tab. VII, figs. 13-15) suggest a rather flattened body, and the author believes this species is intermediate between the two groups. But my strains, which in every other respect are quite like Skuja's form, are almost circular in cross-section.

<sup>2</sup> These grains are represented in a drawing of *M. pellucidum* reproduced by Matvienko (1938, Tab. II, fig. 23) which shows a markedly curved cell and not the typical outline.

cylindrical. The anterior end, when seen from the narrow side, is slightly protruded like a neck.

(2) Length 40–55  $\mu$ , breadth 8–13  $\mu$ , thickness 3–4  $\mu$ .

(3) Striation unrecognizable with the usual optical equipment.

(4) Flagellum about as long as the body.

(5) Nucleus at or behind the middle. Caryosome invisible in the living cell.

(6) Paramylon grains in part large long rectangular rods or elongate rings with a dark central line; the remainder small, rounded to rod-shaped. Pearl-like granules in the back end.

(7) Movement combined with rotation so that both flat sides are visible in turn. Rarely vibrating without rotation.

(8) While *M. obtusum* n.sp. is less protruded at the front and back ends than *M. pellucidum*, the opposite is true of *M. cultellus*.

(9) The strain was reared from material obtained from a pond at Jevany near Prague, the mud being taken from a small bay from which the water had been drained leaving a number of pools. In the enrichment culture *Distigma proteus* was the principal accompanying form.

(10) Putrefaction cultures with starch-containing material and soil were mostly successful. Clone cultures were also easily obtained.

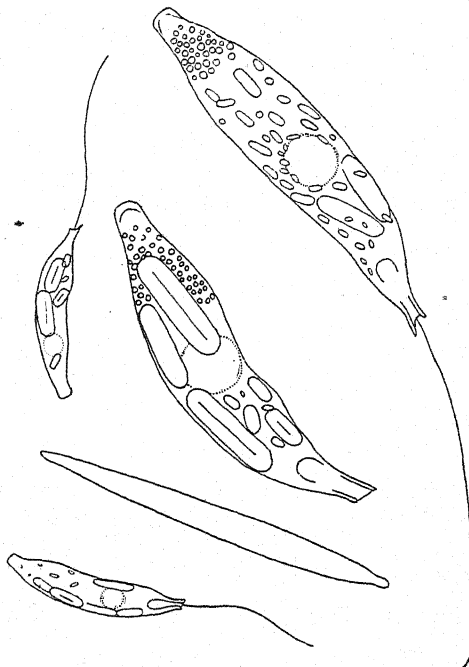


Fig. 16. *Menoidium cultellus* n.sp. One individual is shown from the narrow side to illustrate the marked degree of flattening found in this species.

## 2. *Menoidium obtusum* n.sp. (1936, p. 73, Fig. 8; and Fig. 17 of this paper)

(1) Cells moderately flattened. The outline of the body, as seen from the broad side, is approximately that of a long bean, while the posterior third corresponds to about one-half of a narrow ellipse. In the middle the cell is sometimes a little 'bent', but for the most part only slightly curved. The anterior end is little protruded and does not exhibit a pointed projection, such as is found at the front end in *M. pellucidum*, on the side of lesser curvature. Viewed from its narrow side, the cell appears elongate naviculoid narrowing uniformly to the rounded back end, while the front end is slightly protruded as a neck. The relatively plump form of the posterior end gives the name to the species.

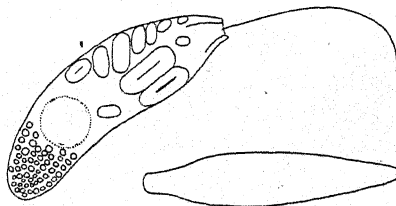


Fig. 17. *Menoidium obtusum* n.sp. Two individuals, seen from the broad and narrow side respectively.

(2) Length 30–37  $\mu$ , breadth 10–12  $\mu$ , thickness 6–7  $\mu$ . The dimensions vary appreciably even in clone cultures, which is probably due to the fact that the cultural conditions are not yet quite in conformity with the needs of the species.



(3) Striae delicate, densely placed, running at an acute angle to the line joining the flagellar and posterior poles.

(4) Flagellum not quite as long as the body.

(5) Nucleus behind the middle, but not near the posterior end, caryosome visible only after fixation.

(6) Paramylon grains in part large, appearing as elongate rings with a dark median line, in part small and elongate to rod-shaped. The large grains are usually arranged in a row adjacent to the curved and thicker dorsal edge.

(7) Movement with rotation around the longitudinal axis. When checked the cells swim with a wobbling movement without rotation, during which the rounded side is directed downwards, probably owing to the weight of the large paramylon grains.

(8) Strains belonging to this species have previously been referred to *M. pellucidum*, but it differs from the type as described by Perty and Klebs in three respects: (1) The posterior end is plumper than shown in the figures of Perty, Klebs, etc. (2) The anterior end is not or scarcely protruded as a neck. (3) The flagellum is longer than is mentioned in the diagnosis of Lemmermann (slightly longer than half the body). The plumper shape of *M. obtusum*, comparing with the diagnoses of Lemmermann and Playfair, is also manifest from the measurements (Pringsheim, 1936, p. 72).

The figure of Playfair (1921, Tab. 7, fig. 1), however, looks different from that of Perty, although in the description of the figure (p. 145) it is claimed to correspond to the 'type'. It is possible to interpret it as an incorrect drawing of *M. obtusum*, but it probably represents another species.

(9) *M. obtusum* cannot be a mere habitat form of *M. pellucidum*, since it was found several times and retained its shape in different kinds of putrefaction cultures.

The material from which *M. obtusum* was raised was derived from: (1) Peaty margins of the Pflegersee near Garmisch in Upper Bavaria. (2) Water with red-coloured mud sent to me by Prof. Rawitscher from São Paulo in Brazil. (3) Ditch water with mud from the Habstein-moor near Hirschberg in Bohemia. (4) Again from the Pflegersee. (5) Mud from Reading.

(10) Putrefaction cultures may be obtained with starch-containing material and soil, but they do not always succeed. Nevertheless, all the strains were preserved for months and even years, and on several occasions clones were isolated.

### 3. *Menoidium bibacillatum* n.sp. (Fig. 18)

(1) Cells strongly flattened. The outline of the broad side of the body is similar to that of *M. obtusum*, but the back end is more or less rectangular with a rounded truncate termination. The form of the front end is about midway between that of *M. obtusum* and *M. cultellus*. The same is true of the shape of the whole cell, of the posterior and anterior parts, and of the cross-section.

(2) Length 36–40  $\mu$ , breadth 10–12  $\mu$ , thickness 5–6  $\mu$ .

(3) Striation very delicate.

(4) Flagellum about as long as the body.

(5) Nucleus central or behind the middle, caryosome invisible during life.

(6) The paramylon grains are characteristic, two being nearly always much larger than the others, the one near the anterior end and the convex edge, the other almost parallel

to it or situated nearer to the back end. They are shaped like very elongate rings with narrow perforations or are solid. The foremost is sometimes considerably larger than the other. Many small grains, in part polygonal, are scattered throughout the cell.

(7) Movement like that of the other species.

(8) This strain might be regarded as a variety, since it does not differ very much from the type or the other species described above.

(9) It was found in a sample collected by Dr L. E. R. Picken from a pool near Cambridge.

(10) Putrefaction cultures with starch.

The genus *Menoidium* therefore includes *M. pellucidum* Perty, *M. obtusum*, *M. bibacillatum*, *M. cultellus*, *M. inflatum* Playfair, *M. distractum* Wermel (1924), etc. *M. acutissimum*, insufficiently described by Playfair in the same paper (1921), is a form of the *Euglena acus* group, related to *Astasia linealis*, as is evident already from the

presence of an eye-spot. Playfair further describes a *Menoidium gracile*, but since neither description nor figure indicate whether the organism is flat, its identity cannot be established. The same is true of the forms described by Skvortzow (1924).

Only one species of *Rhabdomonas* has been found which I had not seen before:

***Rhabdomonas spiralis* n.sp. (Fig. 19)**

(1) Shape irregularly spiral, so peculiar that it might be regarded as teratological, if the organism had not been found in four different localities and always occurred in huge numbers in the self-same form in every subculture.

The cell is rigid and elongate like that of *Rh. costata* (= *Menoidium longum*), but more slender and smaller, slightly flattened, provided with a spiral costa or keel and pointed at the back end. The major part of the body is only faintly curved, but the posterior end is twice bent at right angles. The shape of the body is difficult to describe and likewise difficult to draw or to photograph. The anterior end is relatively broad and is provided at the side of the flagellar insertion with a prominence which recalls the protrusion of some *Menoidia*. Owing to the small size of the cells other details, such as the vacuolar system, etc., are not recognizable with certainty, but there seems to be a relatively large gullet.

(2) Length 14–17  $\mu$ , measured from the flagellar end to the tangent to the curved posterior end, i.e. the largest total length which can be enclosed between two lines.

(3) A striation could not be recognized.

(4) The rather coarse flagellum is slightly longer than the body and sometimes stretched straight out when the organism is at rest or after death.

(5) Situation of the nucleus difficult to establish, probably nearly central.

(6) Paramylon grains ovoid to shortly cylindrical, often arranged in longitudinal series, as in the other species.

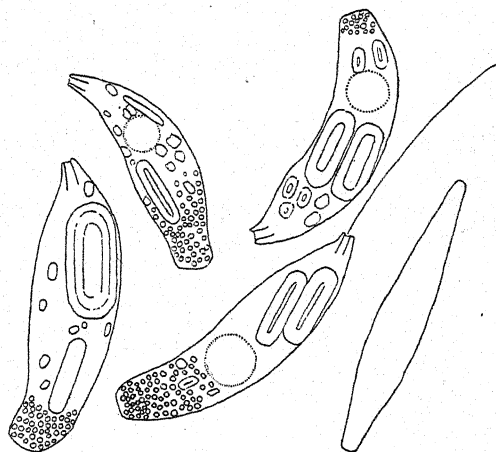


Fig. 18. *Menoidium bibacillatum* n.sp. Most of the individuals contain two large elongate paramylon grains, lying side by side, but in some these show a different disposition or there are more than two of them.

(7) When moving quickly, the cells rotate rapidly about an axis which is oblique to the body and then give the impression of being shaped like a cork-screw; this is, however, only approximately true. The cells generally swim in a lively manner, but they become motionless rather easily.

(8) Skuja (1938-9, p. 112, Tab. VII, figs. 13-15) describes a *Menoidium gibbum* that may be identical or very similar to my species, but it is stated to be more flattened and therefore related to *M. pellucidum*, and to be striated, both of which is not true of *Rhabdomonas spiralis*. Another similar form has been described under the name *Menoidium minimum* by Matvienko (1938, pp. 60 and 69, Tab. II, fig. 24). Unfortunately, his figure is not adequate to leave a possible identity beyond doubt. His short description is clear, but not altogether convincing on this point. Other similar forms which might be confused are not known to me. Spiral curvature is met with in *Menoidium tortum* (Stokes) Senn, which, however, is described as more slender and more regularly curved. The specimen figured by Playfair (1921, Tab. VIII, fig. 7) as *M. tortum* is more like my form than the figure of Stokes reproduced by Lemmermann. I have previously (1936, p. 62) expressed doubts whether Stokes's *Atractonema tortuosum*<sup>1</sup> might not possibly be a stiffened specimen of *Astasia curvata*, but it is equally possible that it may be an inaccurate representation of *Rhabdomonas spiralis*. The matter must await further investigation.

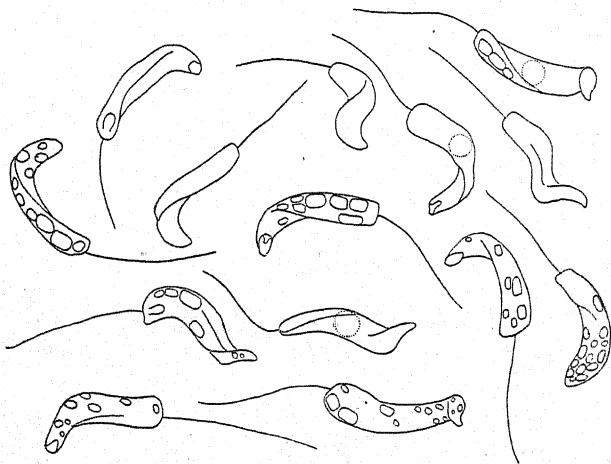


Fig. 19. *Rhabdomonas spiralis* n.sp. Although the individuals are rigid and closely resemble one another in form, numerous drawings are necessary to give a clear impression of the peculiar shape of the species. During movement the actual form of the cells could not be recognized; it then gives the false impression of a regular spiral form.

The species has been referred to *Rhabdomonas*, though it does not altogether correspond to the generic diagnosis given above, since the cells diverge from the rod-like shape. But the cells of the other species are also not straight, but somewhat curved, and the curvature is certainly not in one plane (cf. *Spirillum* and *Vibrio* in the Pseudomonadaceae). In *Rhabdomonas spiralis* there is a stronger manifestation of the spiral tendency which is always present.

(9) First isolated in November 1936 from the mud of a small watercourse near Lunz, subsequently from the mud of a drained fish pond at Jevany near Prague, where it was found again the next year. Afterwards it was detected at Dobříš near Prague, at Franzensbad in Bohemia, in a highland pool above Lunz, and in a garden pool in Cambridge.

(10) Starch-containing material covered with soil affords crowded cultures. Clone cultures are easily obtained.

Among the numerous slightly curved strains of *Rhabdomonas* which have developed in putrefaction cultures and which appeared to belong to *Rh. incurva* Fresenius, two groups

<sup>1</sup> Stokes's species is however pointed at the anterior end and is more slender, larger and more smoothly curved than mine. The name *M. tortuosum* Stokes in Lemmermann's paper is incorrect.

could be differentiated by means of clone cultures. The smaller form may henceforth be regarded as the typical *Rh. incurva*, while the larger may be described as *Rh. incurva* var. *major*. Complete uniformity of size of course does not occur, even within the two varieties. But the great variability mentioned by Lemmermann, according to which *Rh. incurva* is  $16-25\mu$  long, could not be confirmed, neither in a clone, nor in a multitude of strains I collected.

The species, which I believed to be new and termed *Menoidium longum*, has been described by Korshikov (1928) as *M. costatum* in a paper which I overlooked. It should therefore now be called *Rhabdomonas costata* n.comb. The three similar forms of *Rhabdomonas* have the following dimensions: *Rh. incurva* typ.  $13-15:5-7\mu$ , *Rh. incurva* var. *major*  $16-18:6-7.5\mu$  and *Rh. costata*  $28-30:8-9\mu$  (Fig. 20). It is striking that *Rh. costata* has nearly always been found in the numerous enrichment cultures wherever any species of the genus had multiplied. Even when a strain seemed to be different, the secondary cultures mostly contained only the typical *Rh. costata*.

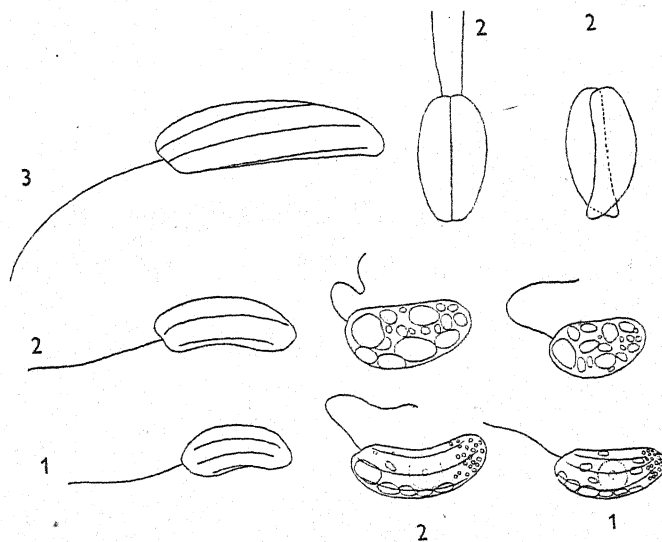


Fig. 20. *Rhabdomonas incurva* and *Rh. costata*. (1) *Rh. incurva* type; (2) *Rh. incurva* var. *major*; (3) *Rh. costata*. It seems that a variety of forms is included in the old *Rh. incurva* Fres., differing in size and in the proportion of length to breadth. The longest and most slender form was referred to as *Menoidium longum* in the previous communication. By means of clone cultures the former *Rhabdomonas* (*Menoidium*) *incurva* has been segregated into two forms, the larger of which occupies an almost middle position between *Rh. incurva* typ. and *Rh. costata*. The figures of division stages in the right upper corner belong to *Rh. incurva* var. *major*. The figures on the left represent diagrammatically the outlines of the three species.

## VII. THE SCOPE OF FURTHER STUDIES

The enrichment and single-cell cultures have proved that there is a large number of saprophytic Euglenineae among which specific distinction is sometimes difficult, but always possible.

As to the interrelationship, the species must at present be treated in different ways. Some can clearly be referred as apochlorotic varieties to definite green species, while for most of them this is not possible. The former are of special interest. It is to be hoped that cultures of others belonging to this group will soon be obtained so that a morpho-



logical comparison between green and apochlorotic forms will be possible. The raising of pure cultures would, moreover, be of value in making possible the physiological investigation of probable parallel forms.

### VIII. SUMMARY

After describing the methods of enrichment appropriate to saprophytic Euglenineae, a procedure for obtaining single cell or clone cultures is described which has proved successful in every case.

The specific characters and the delimitation of the genera *Astasia*, *Distigma*, *Menoidium* and *Rhabdomonas* have become clearer as a result of the study of several new species. Sixteen new species are described and figured. All of them are maintained in culture. I have in my possession at present a collection of thirty-two different forms of apochlorotic Euglenineae.

In conclusion I wish to thank Prof. F. E. Fritsch for his kindly help in the preparation of this paper, especially in rendering it into English.

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## THE MECHANISM OF ELONGATION IN PALISADE CELLS

By R. W. WATSON

(With Plates 3-5, containing Figs. 1-12)

Palisade tissue is composed of elongated usually chlorenchymatous cells which occur adjacent to the epidermis or buried more deeply in the cortex or mesophyll of plant stems and leaves. In its commonest form it is made up of cylindrical cells with their long axes at right angles to the surface of the organ. Arm-palisade, first described by Haberlandt (1880), is distinguished by inward folds or extensions of the walls, and occurs less commonly. A third form, first reported by Pick (1882), is composed of elongated cells with more or less strongly inclined radial walls. In the present investigation an attempt has been made to add to our knowledge of the development of the common or vertical palisade cells and the causes responsible for their formation.

The first scientific observations were published by Thomas (1863). He observed in the leaves of a number of conifers a direct relationship between the illumination and the development of palisade tissue, finding the palisade cells invariably more strongly developed on the brightly illuminated side of the leaf. Confirmatory observations on other forms were made by Frank (1873) and Magnus (1876). Pick (1881, 1882) carried on a comprehensive experimental investigation as a result of which he disagreed radically with the teleological explanations given by Haberlandt (1882). Pick concluded that in the majority of dicotyledons palisade tissue was hereditary, but that in others its presence depended directly on illumination.

Stahl (1883) considered the palisade cells an adaptation to intense light, and concluded that their form was due to expansion in a vertical direction after horizontal expansion had been eliminated by the cessation of vein elongation. Heinricher's observations (1884) led him to deny a direct influence of light in this connexion, while Dufour's experiments (1887) confirmed the views of Pick and Stahl. Clements (1904) criticized Dufour's conclusion in that he used only sun plants.

Eberdt (1887, 1888), while in substantial agreement with Haberlandt's views, suggested that the palisade tissue arose from the combined effects of assimilation and transpiration. Wagner (1892), from work with alpine plants, concluded that transpiration had no bearing on the form of palisade cells. Lothelier (1893), on the other hand, found that, other conditions being equal, leaves grown in dry air showed a more highly developed palisade parenchyma. Eberhardt (1903) raised plants under carefully controlled conditions of light, temperature and relative humidity. Lothelier's opinion with respect to the effect of relative humidity was confirmed, and the statement was made that damp air had much the same effect as shade on the form of the cells. Nordhausen (1903), working with detached beech branches, found that shade buds produced shade leaves and sun buds produced sun leaves regardless of the illumination to which they were subjected during development. Maximov (1929) suggested that Nordhausen's results were explainable by the fact that shade branches have larger vessels and so deliver a more copious

supply of water to the developing leaves. Clements (1904) made morphological studies of a large number of plants and was in substantial agreement with Pick and Stahl. Raunkiaer (1906), working with aerial and submerged organs, concluded that transpiration was not a factor.

Yapp (1912) was the first to measure the lengths of palisade cells in different parts of the leaf lamina. He says: 'All the evidence available suggests that any marked development of these peculiar elongated elements is in some way associated with decreased turgor, rather than with increased turgor.' He inclined to the view that part at least of the effect of light is indirect through its influence on transpiration.

Mothes (1932) grew *Nicotiana rustica* in various modifications of Zinzadze's solution. He shows that in a certain nitrogen deficiency the leaf mesophyll is transformed into palisade-like tissue. He does not give the conditions with regard to relative humidity, light and temperature under which his plants were grown. Attempts on my part to duplicate his results have been entirely unsuccessful. Cain & Potzger (1940) state that in *Gaylussacia baccata* 'the depth of the palisade region shows an orderly arrangement with respect to soil moisture and evaporation, the relationship being direct with soil moisture and inverse to the evaporation'.

A somewhat different aspect of the problem was indicated by two papers published in 1904. Chrysler compared the leaves of several species growing on a sea beach and on the beach of an inland lake. Plants from the maritime region were found to have thicker leaves due to increased development of the palisade tissue. Boodle reproduced the effect on wallflower leaves by spraying them with a solution of sea salt. He suggested that possibly increased turgescence might be the direct cause of their formation.

#### MATERIAL AND METHODS

It is unquestionably true, as first enunciated by Pick (1882), that in many dicotyledons the palisade cell form is hereditary, at least to the extent that it is produced under a very low light intensity. For an investigation of the direct effects of physical factors on the development of palisade cells it is necessary to find a sensitive species in which protoplasmic or genetic control is not so complete as to outweigh the effects of environmental conditions. Moreover, it is highly desirable to have a plant which will tolerate wide light, temperature and humidity ranges, display freedom from dormancy, possess firm smooth long-lived leaves, and be easy to cultivate. After a preliminary investigation involving fifteen more or less unsuitable species, the juvenile shoots of the English ivy, *Hedera helix* L., were found to possess the necessary qualifications.

In order to avoid as completely as possible any genetic heterogeneity in the material, such as might possibly be present in ecotypically different stocks of ivy, it was necessary to employ throughout the investigation individuals derived vegetatively from the same plant. The presence of the numerous anlagen of aerial roots on the juvenile shoots made propagation by layering comparatively easy. Pieces of the shoot about 18 in. long and bearing numerous healthy leaves were layered in flats so that the main stems and bases of the petioles were covered to a depth of about  $\frac{1}{2}$  in. A compost of equal parts of sand, loam and leaf mould proved satisfactory. The flats were kept in a cool, shady place for about a month until the aerial roots had developed into normal absorptive roots. They were then transferred to brighter light. After about two months these root-bearing runners were taken up, and cuts were made with a razor blade about  $\frac{1}{2}$  in. on either side



of the rooted nodes. Each of the small plants thus obtained consisted of a root and a single leaf. These were replanted in flats, and kept in a cool greenhouse for two or three months until several leaves had been developed. They were selected for uniformity, were carefully sprayed with 'Volck' to eliminate 'red spiders', and transferred to  $3\frac{1}{2}$  in. pots. Since the mutation rate is low and since any mutation in meristematic tissue will affect only one clone member, or only a part of its tissues, it becomes apparent that this material is as genetically uniform as it is possible to obtain.

Waxed cards attached to the stems, and correspondingly numbered lead labels placed in the pots, afforded a two-way check, and completely prevented cases of mistaken identity. Each leaf was numbered with permanent ink and measured at the time of the initial transfer. By measuring the leaves at intervals during their development a growth record for each leaf was obtained from which its approximate size at any time could be read. A facsimile growth card is given below. 'B' stands for the basal leaf from which the cutting was grown. The measurements represent lengths in millimetres from the point of junction of the five main veins to the leaf tip.

*Facsimile growth card*

4669	Hedera helix L.						Habitat C										
Leaf number	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12 June 1939	20	5	0.5	0													
18 July 1939	31	23	30	33	18	9	5	2	0								
25 July 1939			30	34	24	17	10	6	2	0							
28 July 1939				34	25	19	14	10	4	1	0						
1 Aug. 1939					25	21	20	15	7	2	0.1	0					
5 Aug. 1939						22	22	20	11	4	2	0					
9 Aug. 1939							22	25	17	10	5	2	0				
13 Aug. 1939								27	22	17	12	6	2	0			
15 Aug. 1939									23	20	15	8	3	0.5	0		
18 Aug. 1939										23	20.5	12	5.5	1	0		

These cards proved invaluable, as from them and from the daily records of environmental conditions the physical factors affecting the growth of each individual leaf could at any time be ascertained.

For clone members which were transferred from one habitat to another a brief record of the conditions to which each had been subjected was kept on transfer cards attached to the pots. These cards bore the letters *A*, *B* or *C* standing for the respective habitats, followed by the dates of transfer.

Leaf development is very slow in the ivy, extending from about one month in bright sunlight to three months in deep shade. For this reason the majority of the plants were grown under natural rather than artificial light. This had the disadvantage that the amount of light received by the plants could not be completely standardized. However, a record of the conditions in two of the natural light habitats, i.e. dark shade (*A*) and light shade (*B*) was kept. These two shade positions were located in a room which was lighted

by three north windows. Dark-shade plants were kept at a distance of 15 ft. from the centre window, while light-shade plants were placed about 2 ft. inside the same window. Dark blinds were kept drawn from 5 p.m. until 9 a.m., so that the plants received north light for 8 hr. per day. The spectral distribution of the energy in the incident light was not measured. Since the light came largely from the north sky its maximum energy probably lay toward the blue end of the spectrum. The Canadian General Electric photo-electric cell used in the light measurements was calibrated by comparison with a Weston cell. From the standpoint of absolute light-intensity measurement this leaves something to be desired, but as a basis for a comparison of intensities the method is sufficiently accurate. Temperature and relative humidity readings are common to the two shade habitats, so that A and B are distinguished only by differences in the light. During the period of leaf development relative humidity determinations were made two to three times daily. The average was 57.3, the maximum 77 and the minimum 34. The wet- and dry-bulb hygrometer used in determining relative humidity was mounted in front of an electric fan which was turned on 5 min. before making the reading. During the same period the temperature varied between 87 and 67° F. Each of the plants received about 20 c.c. of water per day. Light records for the shade habitats are summarized in Table 1.

Table 1. *Intensity of illumination in foot-candles*

Month	Habitat A			Habitat B		
	Av.	Max.	Min.	Av.	Max.	Min.
May	78.4	144	11	731.4	1360	120
June	77.9	100	12	725.7	1280	220
July	54.1	88	16	633.5	1000	240
October	34.2	72	6	365.4	720	48
November	37.8	118	4	239.4	600	20
December	61.7	160	8	420.0	1200	100
Average	57.3			519.2		

Sun leaves were grown during July and August under the brightest conditions available in the garden (habitat C). They probably received from 40,000 to 50,000 foot-candle hours per day. It is difficult to estimate accurately the quantity of light received by plants under natural conditions, so that this figure represents merely an approximation. It was deduced from measurements of the average total solar radiation intensity supplied by the Dominion Weather Bureau whose office was about half a mile distant. The average intensity of the sunlight in foot-candles was obtained with the help of Kimball's conversion factor. 'Kimball arrives at an average value of 6700 foot-candles for 1 g.cal./cm.<sup>2</sup>/min. which is somewhat low for high sun and high for low sun' (Brackett in Duggar (1936), 1, 180). Knowing the average length of day, the average total solar radiation intensity in foot-candles may be obtained by a simple calculation. These values are computed in Table 2.

The sun leaves therefore completed their development in roughly one hundred times the quantity of light received by the leaves developed in dark shade. Records obtained from the Dominion Weather Bureau, taken under comparable conditions, show that the average relative humidity outdoors during the period of leaf development was 70, and the average temperature 64° F. The average humidity was therefore considerably higher and the average temperature somewhat lower than under the conditions in which the

shade leaves were grown. The main difference between the three habitats remains the difference in the quantity of light which increases roughly ten times from A (dark shade) to B (light shade) and ten times from B to C (full sun).

Table 2. *Average total solar radiation intensities, Dominion of Canada Weather Bureau, Toronto*

1940	Average intensity	
	g.cal./cm. <sup>2</sup> /day	Foot-candles/day
January	135	1600
February	162	1725
March	296	2775
April	393	3250
May	390	2975
June	457	3325
July	524	3900
August	436	3750
September	332	2950
October	228	2300
November	95	1100
December	77	950

#### STRUCTURE OF THE SUN AND SHADE LEAVES OF ENGLISH IVY

Throughout the course of experiments of this kind care must be taken to draw conclusions only from plants that have had time to reach a condition of equilibrium with their environment. The apparently anomalous behaviour of plants when moved from one environment to another provides in itself an interesting problem which must await further investigation. When an ivy plant is moved so that it is no longer in equilibrium with its environment, striking changes in the size reached by immature leaves follow. If the plants are removed in summer from the comparatively humid atmosphere of the greenhouse to the brighter and drier conditions in the garden a few very small leaves, less than one-half the size of a normal sun leaf, are developed. If well-grown sun plants are transferred to dark shade conditions the developing leaves expand to an extreme size, reaching a surface development five or more times the area of leaves developed by a plant in equilibrium with dark-shade surroundings.

Leaves in full sunlight completed their development from rudimentation to mature size of the lamina in about 4 weeks. Thus leaf 7 of plant 4669 (p. 6) developed in the garden in full sunlight (C) was 2 mm. long on 18 July and had completed its expansion of 27 mm. on 13 August. In dark shade the expansion of the lamina is several times slower. Thus leaf 4 of plant 4754, grown in dark shade (habitat A), was 0.5 mm. long on 24 May, and had not reached its mature size of 20 mm. until about 9 August, when it was more than 11 weeks old.

The reaction of an ivy plant to the condition of illumination makes it very suitable as material for the study of palisade-cell development. Fig. 1 shows the structure in cross-section of a leaf from dark shade. This leaf required about 3 months for complete development. During this time it received an average of about 70 foot-candles for an 8 hr. day. The unstained section was photographed in water shortly after it was cut, so that most of the cells were still living.

The upper epidermal cells have thin, straight or somewhat inclined radial walls, and thin tangential walls. Ordinarily the cells contain large pale chloroplasts. The cuticle is

approximately  $0.5\mu$  thick. Cylindrical or prismatic palisade is not present. In a few cases the long axes of the thin-walled subepidermal cells are parallel to the leaf surface, but usually these cells are isodiametrical. Intercellular air spaces are small. In the underlying layer the cells are similar but fewer, more rounded and with larger air spaces. Cells in the mid-mesophyll show a tendency to elongate parallel to the leaf surface. Chloroplasts are clearly concentrated in the upper half of the leaf. In the spongy layer irregular arms with intervening large air spaces extend from cell to cell. The stomata are restricted to the lower epidermis. The entire thickness of such a leaf averages about  $190\mu$ . A cross-section of a leaf from light shade resembles fairly closely the one described for dark shade.

A similar unstained section of a sun leaf is represented in Fig. 2. This leaf was developed in full August light, and therefore received an average of about 3500 foot-candles for a 14 hr. day, or in the vicinity of 50,000 foot-candle hours per day.

Both radial and tangential walls of the upper epidermal cells are substantially thicker. Chloroplasts are not present in the epidermal cells. Accurate measurements show that the cuticle varies from 2 to  $3\mu$  in thickness, i.e. it is four to six times as thick as that of a dark-shade leaf.

The two upper layers of mesophyll cells have become cylindrical and form the palisade tissue. In the uppermost layer air spaces are smallest. In the underlying layer the spaces become wider, and widen downward. Even in the third layer some of the cells have their long axes vertical to the leaf surface. In all three layers the walls are thicker than in the corresponding cells in the shade leaf. Chloroplasts are abundant throughout the mesophyll, but are most numerous along the side walls of the palisade cells. In the uppermost layer they are always absent along the wall adjoining the upper epidermis. As in the shade leaf the irregular cells of the spongy parenchyma are connected by extensions of their walls, and separated by large air spaces. In the lower epidermis the radial and tangential walls of the cells are thinner than in the upper epidermis, but still considerably thicker than in leaves from dark shade. The entire thickness of this leaf averages  $265\mu$ . Usually a sun leaf is about 1.5 times as thick as a leaf developed in shade.

The most conspicuous difference between these two contrasted leaf types is the difference in the development of the palisade tissue, which is absent in leaves from both dark and light shade, and consists of two well-developed layers in the sun leaf. The increased thickness of the sun leaf is mainly due to the elongation of the palisade cells. In the historical introduction the long dispute over the manner in which light effects this elongation was reviewed. The experiments recorded in this paper were designed to increase our knowledge of this vexed problem. In order to avoid the complication introduced by the changing size of the growing lamina it was decided to use for experimental purposes only young leaves which had completed their development in area.

In a first attempt to determine the direct effect of an increase in light on the expansion of the uppermost mesophyll cells a mature light-shade leaf was transferred directly to full sunlight. The entire thickness of the leaf before the transfer was about  $180\mu$ . The uppermost layer of the mesophyll was composed of approximately isodiametrical cells, and this was true also of the second layer.

After exposure for 1 day to the intense sunlight of early July dark patches were present in several parts of the lamina. It was noticed that the epidermis over these patches had



retained its lustre, so that the injury seemed to be in the mesophyll. On sectioning the leaf 4 days after its transference it appeared as shown in Fig. 3. The cells of the upper epidermis were apparently still alive although no critical tests to prove this were made. The cells of the uppermost mesophyll layer had been killed over wide stretches *except in proximity to the veins*. The protoplasts in these cells had become shrunken and brown and therefore appear darker in the photograph than those of the living cells beneath. Moreover, the walls can be seen to be buckled and crumpled in many places. Every cell of the next underlying layer of mesophyll was turgid and healthy, and this condition held for all of the many sections of such leaves examined.

Several conclusions follow from this simple experiment. The fact that the subepidermal cells were unharmed in proximity to the veins suggests that the cells were killed by drying out. The continued vitality of the upper epidermal cells indicates that they are in some way protected against death from desiccation. Iljin (1935) was able to demonstrate this property in the epidermal cells of the red cabbage. By first cautiously plasmolysing he was able to dry the cells for weeks in an exsiccator and then to retain them alive after careful deplasmolysis. The layer of cells below the uppermost mesophyll layer was certainly unharmed, pointing to a strongly localized water deficit in the uppermost mesophyll layer.

Subsequent changes in the structure of this leaf were slow. They first became apparent after several weeks. Fig. 4 shows the structure in cross-section of the same leaf after 3 months in the open. The changes include (1) the death and collapse of the upper epidermal cells, (2) the complete collapse of the uppermost layer of mesophyll cells, (3) the expansion to the cylindrical palisade cell form of many of the cells previously forming the middle of the leaf, and (4) the initiation of cell division and the formation of cross-walls in the upper parts of these cells.

During these changes the leaf had increased about one and three-quarter times in thickness, although the area had remained the same. Strong pressure, apparently due to an increase in turgor in the living mesophyll cells, had crushed the upper epidermal and the rows of dead mesophyll cells tightly against the cuticle.

This apparent increase in turgor pressure suggested an accumulation of osmotically active substances in the cells, a suggestion which was proved correct by osmotic value determinations on other leaves treated similarly. Experiments to explain this increase were then inaugurated.

An investigation to determine changes in the starch-sugar ratio was first undertaken. The leaves used were transferred gradually from dark shade to brighter light to avoid the damage occasioned by too sudden a transfer, and preparations were made to show the starch content of the cells at various times during the transfer.

The hand sections that had been used in all previous work proved unsatisfactory, since their unevenness made the task of preparing successful starch mounts difficult, and a method was devised for making microtome sections of the living leaves.

In order to obtain a series of sections at different times from the same leaf a punch was used which removed a circular 5 mm. disk. The leaf disk was mounted between two pieces of desilicified pine wood. The pine block was cut so that one piece was L-shaped, with a smaller block fitting into the open side of the L. The disk was placed so that it was held at right angles to the jaws of a sliding microtome clamp, and the knife was mounted strongly inclined to the block. In order to obtain one layer of undamaged cells

it was necessary to cut sections of sun leaves  $50\mu$  thick. For shade leaves this had to be increased to  $60\mu$ . These sections were uniform throughout their length. In 15 min. it was easy to prepare fifty sections. The sections were first transferred to a dish of neutral water, from which they were removed one by one to 20 c.c. of fresh neutral water to separate them from the pine shavings. After 1 min. under reduced pressure produced by a filter pump enough air had been removed to allow them to sink. Thirty seconds in a hand centrifuge was then sufficient to remove the rest of the air from the intercellular spaces.

To prepare the starch mounts fresh sections were transferred to a solution of 1 g. of iodine in 100 c.c. of 1 % potassium iodide solution and left there for at least 2 hr. They were removed singly to a chloral hydrate solution in a small watch-glass (8 parts chloral hydrate to 5 parts water), where they remained for 1 min. Glycerin, to which a few drops of a strong tincture of iodine in 95 % alcohol had been added, served as a mounting medium. Sometimes the original appearance of such a preparation would be retained for 10 days. The photographs were taken immediately after the mounts had been made.

The starch content of a leaf grown for 5 months in an average illumination of 80 foot-candles, an illumination not far above the critical for the shade leaf, is shown in Fig. 5. This leaf received about 20 foot-candles more than those grown in dark shade, but presents the same appearance except that the starch grains are slightly larger and somewhat more numerous. Even when swollen with chloral hydrate their size is very small. Moreover, they are slightly smaller and fewer in the cells forming the uppermost layer of the mesophyll than in the cells just beneath. Some of the grains are large enough to reveal their crescentic form.

Fig. 6 shows the starch content of such a leaf 5 days after transfer to light shade. In November when this transfer was made the average illumination in habitat B had fallen to about 240 foot-candles. The cells in the uppermost mesophyll layer are filled with starch to such an extent that they appear solid black in the photograph. Synthesis of starch under these conditions is obviously more rapid than hydrolysis. Ordinarily starch accumulation is not rapid in such a low illumination intensity, so that we must assume hydrolysis to be exceedingly slow. The cells of the uppermost mesophyll layer contain approximately as much starch as those below them. At least no difference in the starch content can be detected visually. The accumulation of the products of photosynthesis in the upper half of the leaf under these conditions is striking.

The same leaf after 10 days in light shade (B) is shown in Fig. 7. The starch content of the upper layers of mesophyll has fallen considerably, the change being slightly greater in the subepidermal layer than in that just below it. In Fig. 8, representing the same leaf, 15 days after the transfer, a further difference between the starch content in the cells of the uppermost and the next underlying mesophyll layers is apparent. After 20 days in B light the difference is still conspicuous, and elongation of the cells has begun. This elongation is considerably further advanced 40 days after the transfer as shown in Fig. 9.

The extreme slowness of the expansion in this series is partly due to the low illumination intensity in light shade (B) in November. An interesting observation emerges here. Although leaves which undergo their complete development in habitat B show only isodiametrical cells below the upper epidermis, leaves grown in habitat A, and transferred after completing their growth in area to light shade (B), exhibit an elongation of

the uppermost mesophyll cells. Fig. 11 shows the extent to which this palisading of a mature dark-shade leaf may go after 80 days in the greenhouse from 5 September to 26 November. Since the transfer from dark shade to bright light was gradual the leaf tissues have not been damaged, but the previously subglobose cells have in some cases tripled their original length to assume an accentuated palisade cell form. Moreover, the elongated cells have lost most of their starch, in sharp contrast to the isodiametrical cells below them.

Nothing in the literature nor in the present investigation provides any basis for a suggestion that these pronounced differences in starch content are due to differences in the assimilatory activity of the various cells. In fact the opposite is the case, the suggestion being that, under the light intensities employed, greater photosynthetic activity occurs in the uppermost layer of mesophyll. We are therefore led to adopt the hypothesis of a change in the starch-sugar ratio to account for these discrepancies.

Molisch (1921), Ahrns (1924), Spoehr & Milner (1939) and others have shown that the water content of leaf cells profoundly affects their rate of starch hydrolysis. There are no stomata in the upper epidermis of the ivy leaf, but the inference that the differences described above might be due to cuticular transpiration seemed obvious, and the verification of a strongly localized water deficit in the cells immediately below the upper epidermis as suggested by Fig. 3 supported this view.

A simple experiment was designed to test this hypothesis. A shallow circular glass cell about  $\frac{1}{2}$  in. wide, and  $\frac{1}{4}$  in. deep was affixed to the upper surface of a sun leaf with a mixture of beeswax and vaseline. Two small holes  $90^\circ$  apart had been bored through the glass sides of the cell. The top was sealed with a cover-glass, and into one of the holes a tube was passed, connected in such a way that air could be circulated through the cell. The other hole served as an exit. The illumination at the leaf surface was fixed at 1000 foot-candles under a tungsten filament bulb, and a water filter 4 cm. deep was arranged between the light and the leaf. The light measurements were made with a Canadian General Electric photo-electric cell and a microammeter, calibrated as before by direct comparison with a Weston meter.

A sun leaf was exposed for 4 days to light periods of 16 hr. per day with a continuous stream of air saturated with moisture at room temperature passing through the cell. The air current was at the rate of roughly 100 c.c./min. for the duration of the experiment. Under these conditions a precipitate of water drops deposited inside the cell during the dark periods and slowly disappeared when the light was turned on, showing that the air passing over the cuticle must have been slightly below the saturation point during the periods of illumination. The starch distribution after this treatment is shown in Fig. 12. An unnaturally heavy accumulation of starch is seen to be uniformly distributed in the uppermost mesophyll layers.

A similar leaf of the same age developed under the same conditions, and at the same height on the stem was allowed to assimilate under conditions identical with the preceding except that dry air was passed through the glass cell affixed to the epidermis. The relative humidity of this air was almost zero. From the compressed air tap it passed through a glass cylinder 20 in. long packed with calcium chloride, through a washing bottle fitted with a Jena glass scrubber containing 4 in. of C.P. sulphuric acid, and finally through three U-tubes packed with calcium chloride before entering the glass cell affixed to the leaf surface. The final U-tube maintained an approximately constant weight,

so that the air above the cuticle contained from 0.14 to 0.25 mg. of residual water per litre (McPherson in Hodgman, 1935, p. 865). Fig. 10 shows the distribution of starch in a leaf exposed to dry air in this way. There is a conspicuous diminution of the starch content in the cells of the uppermost mesophyll layer in striking contrast to the great accumulation in the second layer of palisade cells. Since the light conditions were the same in both experiments the direct effect of the lighting cannot be held responsible for the results. The diminution of starch in the upper palisade cells of the second leaf was presumably due to the drying effect of the air that passed over it. The abundance of starch in the second layer of palisade cells is a further indication of the strictly localized character of the water deficit produced in the mesophyll by cuticular transpiration.

A second set of experiments was arranged as follows. Young clone members were transferred to jars containing 250 c.c. of Shive's solution with the following formula:

$\text{KH}_2\text{PO}_4$	...	...	50 g.
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	...	...	25 g.
$\text{Mg}(\text{SO}_4) \cdot 7\text{H}_2\text{O}$	...	...	75 g.
Distilled water	...	...	20 l.
$\text{Fe}_2(\text{SO}_4)_3 \cdot \text{aq.}$	5 %	...	5 c.c.
			Conc. 0.75 %

They were placed in a dark room and exposed to artificial light for 16 hr. per day under a 1000 W. tungsten filament bulb. The temperature in the room varied between 15 and 20° C. and the average relative humidity was about 50. The illumination intensity in the position of the developing leaves was approximately 1000 foot-candles. Leaves developed under these conditions possessed two rows of elongated palisade cells, each about twice as long as broad. The palisade development resembled that of a sun leaf (Fig. 2). Under identical conditions, except that the light period lasted only 4 hr. per day, the leaves became just as large but possessed only isodiametrical subepidermal cells. Thus it was shown in dry air that elongated palisade cells were produced provided that the period of exposure to a light intensity of 1000 foot-candles was sufficiently prolonged.

For the corresponding experiment with saturated air the whole plant with its container of nutrient solution was placed in a cylindrical glazed earthenware crock 19 in. tall and 15 in. in diameter outside measurements, with walls about  $\frac{1}{2}$  in. thick. The cover consisted of  $\frac{1}{4}$  in. plate glass provided with five ground holes for rubber stoppers opening just within the inner periphery of the crock. A water-tight seal with the ground upper edge of the crock was made with a mixture of beeswax and vaseline. The crock was sunk in a water-bath maintained at a temperature of 20° C. with an ordinary variation of not more than  $\pm 0.1^\circ$  C. On a few occasions during the 6 weeks of the experiment the temperature varied as much as  $\pm 0.4^\circ$  C. This immersion in the constant-temperature tank was necessary to prevent the abnormal rise in temperature that would otherwise have been produced by the strong light shining into the closed chamber. The temperature in this case was much more constant than with the experiment just described in the dry air of the room, but 2½ years of experimenting with English ivy indicate that the variation of about 5° C. had no effect on the results.

The five rubber stoppers in the plate-glass cover were made use of as follows. Two of them accommodated glass tubes carrying air saturated with water at the temperature of the tank. Another contained a thermometer and a fourth an exit tube. Through the fifth



stopper three tubes entered, one leading to the bottom of each of the three culture jars containing the experimental plants. By this arrangement the solutions could be removed with the aid of a vacuum pump, and new Shive's solution could be added without removing the plate glass. The plants could thus be left undisturbed throughout their growth period. Water filled the copper tank to a depth of about 1 in. above the plate-glass top. A sensitive mercury thermostat, a heating coil, a coiled copper tube through which tap water was passed, and a mechanical stirrer allowed the temperature to be maintained uniform throughout the tank. To eliminate the varying illumination occasioned by the continual ripples formed by the stirrer a large crystallizing dish in which a constant level of 7 cm. of water was maintained was mounted  $\frac{3}{4}$  in. above the circular top of the chamber, the bottom of the crystallizing dish being immersed in the water of the constant-temperature tank. The same 1000 W. bulb used in the former experiment was suspended 7 in. above the water surface. This provided approximately the same intensity of illumination, measured with the Canadian General Electric photo-electric cell, as in the previous experiment. To make this measurement the photo-electric cell was mounted on a movable stand in the growth chamber, and wires were led out through the air exit tube to a micro-ammeter on the desk. Measurements in various parts of the chamber showed that the illumination intensity varied but that an average value of 1000 foot-candles for the position of the developing leaves was as accurate as any of the other light measurements. A current of saturated air with a rate of flow of  $7.5 \pm 1.5$  l. per minute passed continually through the chamber during the 6 weeks' growth period. The light was turned on for 16 hr. per day. Under these conditions the leaves which came to maturity produced only isodiametrical cells in place of palisade.

The experiment was repeated with a Mazda Bipost tungsten bulb and an improved reflector which increased the intensity of illumination in the chamber approximately four times. Where previously the leaves had grown under an average illumination of 1000 foot-candles, the average intensity after the substitution of the improved bulb was about 4000 foot-candles. A maximum intensity of 6040 foot-candles was measured in the centre of the growth chamber at the bottom. At the upper outer edge there was a minimum of approximately 3000 foot-candles. Toward the centre of the chamber, where the leaves which reached maturity developed, the average intensity was about 4000 foot-candles. Again the growth period lasted for 6 weeks, and the periods of illumination for 16 hr. per day.

Leaves developed in this stronger light in saturated air, and supplied with an abundance of water from Shive's solution, were no larger in area than leaves developed in full sunlight. They became thicker ( $350\mu$ ) than leaves developed under any of the other conditions utilized. Three distinct layers of palisade were present, with the cells in each layer about twice as long as broad. The longest cells were those in the uppermost layer. From the above experiments with a 16 hr. day and a light intensity of 1000 foot-candles, the dry air which brings about a diminution in the starch content of the cells will produce elongation, while under the same lighting conditions, but with saturated air, the elongation does not take place. If the illumination is increased four-fold, however, elongation of the cells does take place despite the saturation of the air surrounding the leaf.

In seeking some explanation of the mechanism through which cuticular transpiration might produce the conspicuous changes in the starch-sugar ratio described above, attention turned to the vacuolar pH of the mesophyll cells. In this connexion Scarth (1932)

says: 'As regards the cells of the leaf other than the guard-cells no definite results were obtained as to their pH or changes therein. The indications are that it is fairly constant. Acidic indicators, in particular brom-cresol purple, were allowed to accumulate in the leaf-cells of *Anthericum* by setting cut leaves in a solution of the dye, but no appreciable difference in coloration was noted, according as the leaves were kept in light or darkness.'

In our investigation the technique of Small's Range Indicator method was adapted for use on the ivy leaf. Since wide departures from Small's method became necessary in fitting the technique to the material, the changes are described below.

After repeated tests under different conditions it was found that the sulphon-phthalein dyes of the Clark and Lubs's list would not accumulate in the cells of leaf sections so that they had to be omitted. This left only diethyl red, methyl red and benzene-azo- $\alpha$ -naphthylamine. The exact methods used in the preparation of solutions of these dyes follow.

Diethyl red was obtained in the form of the dry British Drug Houses Indicator. Of this powder 0.1 g. was ground in a glass mortar for 10 min. with 5 c.c. of *N*/20 NaOH solution. Analar sodium hydroxide was used. Twenty c.c. of freshly distilled water from a Barnstead still were added giving a 0.4% aqueous stock solution. This proved too concentrated for use as a vital indicator. A 0.1% solution was prepared by dilution with distilled water. On attempting to adjust the pH of this final solution to neutrality with *N*/200 NaOH and *N*/200 HCl it was found impossible to fix the end-point at pH 7. The solution as finally used had a pH of 7.7 which it maintained.

Methyl red was prepared similarly. Of the dry powder (National Aniline Co.) 0.2 g. was ground for 5 min. in a glass mortar with 14.8 c.c. *N*/20 NaOH solution. The sediment was retained. 35.2 c.c. of freshly distilled water were added while stirring. The pH of this solution is high (8.5-9.0). Again it was impossible to lower its pH to 7.0 without the addition of too much *N*/20 HCl, so that it was filtered and kept as a stock solution at pH 7.5. By diluting with distilled water a 0.1% sodium-mono-aqueous methyl red was obtained which was easy to neutralize. After adjustment the pH remained about 7.0.

Dry benzene-azo- $\alpha$ -naphthylamine is very slightly soluble in cold water. Various ways of dissolving this dye were tried, none of which was completely satisfactory. Small gives as the British Drug Houses formula '0.01% hydrochloride dissolved in 30% alcohol'. After several trials 0.01 g. of the dry powder was ground in a glass mortar with 1 c.c. *N*/20 HCl for 5 min. Forty-nine c.c. of 30% alcohol were then added and the solution allowed to stand for several hours. This gives a 0.02% dark red solution in approximately 30% alcohol. For comparison a 0.1% solution of the hydrochloride in 20% ethyl alcohol was also prepared. The concentration of this latter solution was found to be more satisfactory, so that it was used by preference. On attempting to adjust the pH to neutrality so much of the dye was precipitated as to render the solution worthless. It was therefore used with the pH of 2.8 which it possessed after preparation.

The useful ranges of these dyes are given in Small (1929, p. 46). The technique utilized by Small in the accumulation of the indicator solutions did not work well with sections of ivy leaves. The cells in the sections of the dark-shade leaves were so delicate that they usually died before the dyes had accumulated. Even with the sun-leaf sections the method proved tedious. The following improvement was finally employed (Scarth, 1926).

The sections were first plasmolysed for 10 min. in slightly hypertonic sucrose solutions. Half-molar sucrose was used for the shade leaves and nine-tenths molar sucrose for the

sun leaves. After plasmolysis the sections were transferred with the adhering sucrose to a dry slide. A cover-glass, supported on bibulous paper so that it did not touch the sections, was added, and a drop of the indicator solution placed at the side of the cover-glass. It was thus possible to watch the deplasmolysis, and the rapid accumulation of the indicator in the vacuoles. By test it was found that the colours thus produced in sun-leaf sections were identical with those following a prolonged immersion in the indicator solution. Under such conditions the cells in sun-leaf sections were extraordinarily long-lived. They have remained capable of plasmolysis and deplasmolysis for 100 hr. in neutral water. They will live for many hours in the indicator solutions, whereas sections of dark-shade leaves deteriorate rapidly even in water.

Another test was undertaken to determine whether the cells were damaged by the hypertonic sucrose solutions. Since no difference in the colours finally attained was apparent in sections which had been passed up through a series of sucrose solutions at one-tenth molar intervals from 0.1 molar sucrose, it was assumed that the cells had not been damaged by the stronger solutions. The sucrose solutions had a  $pH$  a little below neutrality (6.6–6.8). An attempt to adjust the  $pH$  to exact neutrality with  $N/20$  NaOH was followed by the slow decomposition of the sugar.

The mesophyll cells of leaves grown in dark shade always remained colourless (i.e. yellow) with diethyl red, indicating a vacuolar  $pH$  greater than 5.9. This result was obtained so many times without a single exception that there can be no doubt of its accuracy. Moreover, if the solution in which the sections were lying was acidified with gaseous  $CO_2$  the cell sap would turn red, indicating that the dye had accumulated in the cell sap but was on the alkaline side of its colour range. Unfortunately, the indicators available did not allow an exploration of the range between  $pH$  5.9 and neutrality. Attempts to decide this question by the use of neutral red were not successful. Neutral red is not a sensitive indicator, and is very strongly adsorbed by the colloids present in the cell sap. It was therefore impossible to determine the upper limit of vacuolar  $pH$  in dark-shade leaves with the available indicators.

On applying the same methods to a sun leaf a striking contrast appeared. If the sun leaf had been standing in darkness for several days the vacuolar sap of the mesophyll cells stained uniformly red with diethyl red and methyl red, indicating a  $pH < 5.2$ . Only once in dozens of trials was a red colour obtained with benzene-azo- $\alpha$ -naphthylamine. In spite of the low  $pH$  of the benzene-azo- $\alpha$ -naphthylamine solution the cell sap in almost every sun-leaf cell accumulates this dye in its yellow form. The vacuolar  $pH$  of the mesophyll of a sun leaf left in darkness for several days may therefore be set with some conviction between 4.8 and 5.2, i.e.  $5.0 \pm 0.2$ . After a full day in summer sunlight a gradation in the colours assumed by the cell sap in various cell layers of a sun leaf is seen. The best results are obtained with diethyl red. The vacuoles of palisade cells viewed through clear spaces devoid of chloroplasts stain light pink. As one proceeds toward the lower epidermis the colours darken to deep red. If the colour of the dye under this condition is considered to be an accurate indicator of hydrogen-ion concentration, the  $pH$  of the sap in the palisade cells in sunlight must be about 5.6.

Confirmation of the difference in  $pH$  between sun and shade leaves came after learning of a technique developed by Küster (1912) for the accumulation of acid dyes in the cells of undamaged leaf laminae. If the petiole of a dark-shade leaf is cut off under a concentrated (0.8%) solution of brom-cresol purple, and the leaf is left in dim light for several days to absorb the dye, the mesophyll cells adjoining the vein endings become

blue indicating a  $pH$  greater than 6.2. If the same experiment is tried with a sun leaf the cells remain colourless (yellow), indicating a  $pH$  less than 5.9.

The changes in vacuolar  $pH$  undergone when a dark-shade plant is transferred to light shade have been followed in two different leaves. In the first 5 days in which these leaves were exposed to the brighter illumination no change in vacuolar  $pH$  could be detected. In every test the vacuoles remained colourless with diethyl red. On the seventh day a very faint pink was observable, and after 10 days the cell sap stained red with diethyl red and methyl red, but yellow with benzene-azo- $\alpha$ -naphthylamine indicating a  $pH$  of  $5.0 \pm 0.2$ . It is thus shown that the progressive disappearance of the starch from the palisade cells which was described in the earlier part of this paper does coincide in time with a fall in the vacuolar  $pH$ .

#### SUMMARY AND DISCUSSION

The factors which bring about development of palisade tissue in leaves may be classified under two headings: (1) those that produce vacuolation and enlargement of the cells, and (2) those that determine the final cylindrical shape. The juvenile form of the English ivy is an eminently suitable plant for the investigation of these factors since it is easy to grow and propagate, is tolerant of a wide range of environmental conditions and responds to strong light by the production of prominent palisade tissue in its leaves. The same plant when grown in shade produces leaves without palisade tissue of any kind. The bearing of the evidence obtained in the present investigation concerns the factors included in (1) above.

Plants with full-grown leaves were removed from the light-shade habitat (B) to the garden (C) where they slowly developed palisade cells. One day after the transfer the layer of cells underneath the upper epidermis was killed with the exception of those cells in close proximity to the veins. The importance of this phenomenon lies in the fact that since the dead cells are never adjacent to the source of water supply the evidence is that their death was due to desiccation through the cuticle of the upper epidermis. The localization of this desiccation is indicated by the fact that the cells immediately below were uninjured; and the fact that death of the epidermal cells occurred only at a later date is in harmony with Iljin's discovery (1935) of the remarkable resistance of epidermal cells to death by drying. When plants were moved gradually from dark to light shade and then to the greenhouse no killing of the cells ensued.

It was found that less accentuated palisade tissue could be obtained by merely moving plants with mature leaves from the dark- to the light-shade habitat, and in such plants the successive stages in the process were carefully studied.

In these leaves the first noticeable change was a pronounced accumulation of starch in the upper layers of the mesophyll, due presumably to increased photosynthesis in light shade. Later, however, the starch content decreased, particularly in the uppermost layer of cells, that is in those cells exposed to the strongest light. At the same time the osmotic value of the cells rose. The indications from these phenomena are that the increased light intensity had two effects: (1) to increase photosynthesis, and (2) an effect which was later in manifesting itself to modify the starch-sugar ratio, and so bring about a high concentration of sugar and therefore a high osmotic value in the cells. Subsequently the cells absorbed water and palisade tissue was formed. It was found also that the vacuolar  $pH$  of the sub-epidermal cells decreased from above 6.2 to about 5. This change in  $pH$  was accompanied by a lowering of the starch-sugar ratio.



Confirmation of the theory that desiccation had a causal relationship to the change in the starch-sugar ratio was obtained from experiments where leaves under identical conditions with respect to light and temperature, as well as with respect to previous experience, age and height on the stem had portions of their upper surfaces subjected to the action of moist and dry air respectively. The leaf treated with dry air showed a diminished starch content in the upper mesophyll layer, while the moist air produced no such effect.

A second experiment where whole plants with their roots in nutrient solution were kept for some weeks, one set in saturated and the other in dry air, carried the demonstration still further. In this case, the duration of the period of illumination being sufficient, the plants in dry air produced a double row of palisade cells while those in moist air produced none. In all these experiments with dry and moist air artificial light was used and carefully regulated, so that there is no doubt that the starch-sugar changes and the formation of palisade cells were due to dryness and not to a difference in light intensity.

That light does have a direct influence in the formation of palisade cells was, however, indicated by a further experiment, where it was found that with a light intensity four times as great palisade tissue developed even in saturated air. Experiments are now being carried on to ascertain whether this direct effect of light is not an effect on the shape of the cells rather than on their vacuolation.

The indication from experiments so far completed seems to be that in the case of sun leaves of English ivy the uppermost cells of the mesophyll become more or less desiccated through cuticular transpiration, and as a result the starch-sugar ratio shifts, bringing about a greater concentration of osmotically active substances in the vacuoles. This in turn causes the increased vacuolation responsible for the expansion of the cells.

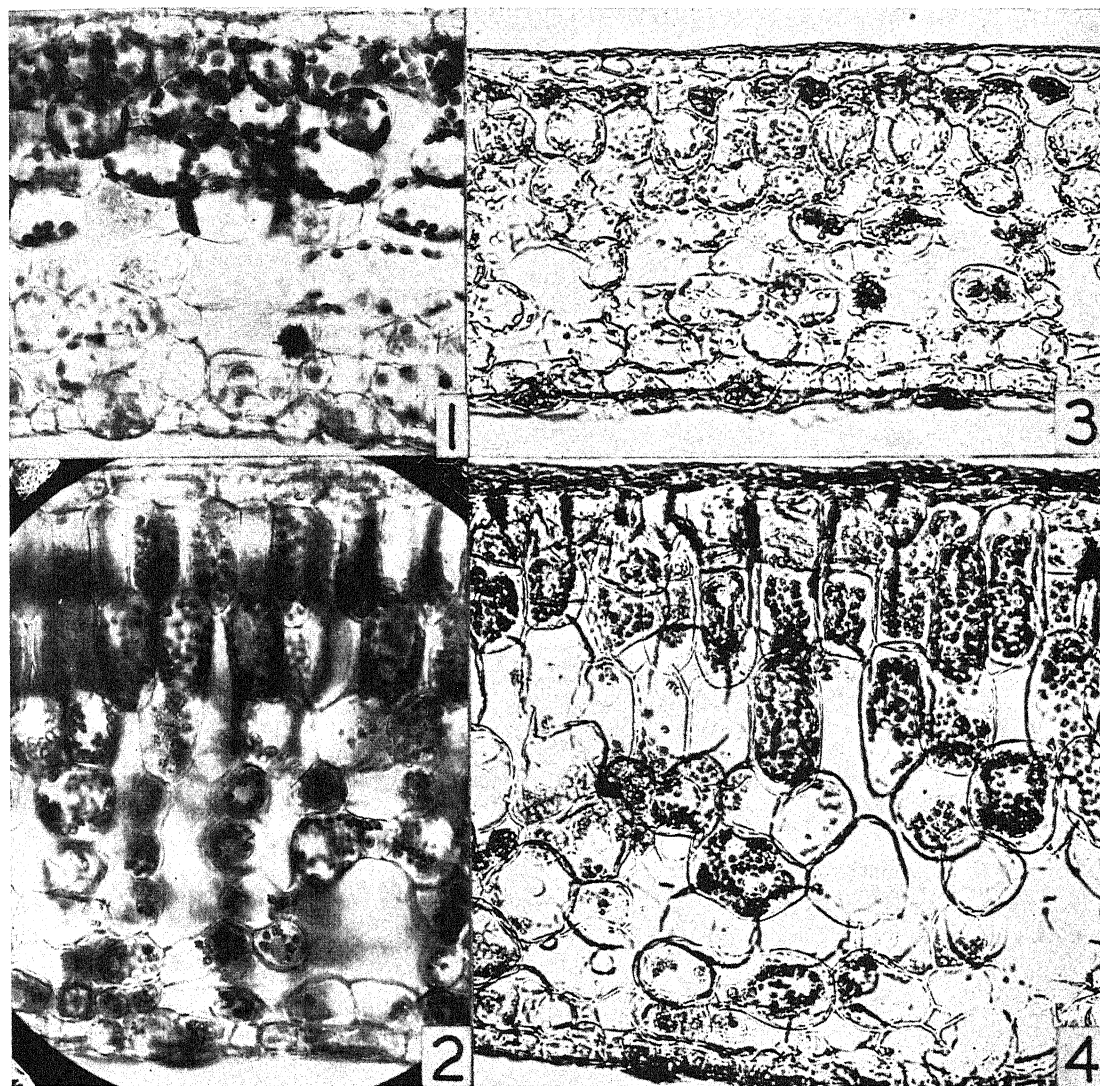
It is important to note that the series of changes leading to active vacuolation and the formation of palisade tissue took place not only when full grown leaves were transferred to conditions of increased light intensity as in the earlier experiments, but also when leaves grew from unfolding to maturity under the proper conditions of light and moisture, as in the experiments with artificial illumination. It is in the latter circumstances that the formation of palisade tissue takes place in nature.

In conclusion I wish to express my gratitude to Dr H. B. Sifton for suggesting the problem and for his help throughout the investigation. I should also like to thank Dr D. H. Hamly for his kindly help with the photomicrographs.

#### EXPLANATION OF PLATES 3-5

All the figures represent cross-sections of leaves of the English ivy, *Hedera helix* L. In Figs. 5-12 inclusive the starch grains are stained with iodine and swollen with chloral hydrate.

- Fig. 1. Dark-shade leaf grown in an illumination of 70 foot-candles for an 8 hr. day: living cells, unstained.  $\times 260$ .
- Fig. 2. Sun leaf grown in full August light: living cells, unstained.  $\times 260$ .
- Fig. 3. Light-shade leaf 4 days after transference to full July sunlight, showing dead subepidermal cells: mounted in glycerin, unstained.  $\times 260$ .
- Fig. 4. Appearance of the same leaf shown in Fig. 3, 3 months after transfer to full daylight: mounted in glycerin, unstained.  $\times 260$ .
- Fig. 5. Starch content of a leaf grown in an illumination of 80 foot-candles for an 8 hr. day.  $\times 260$ .
- Fig. 6. Heavy starch accumulation in a dark-shade leaf 5 days after removal to light shade.  $\times 260$ .
- Fig. 7. Same leaf as in Fig. 6 showing the lowered starch content 10 days after the transfer.  $\times 260$ .
- Fig. 8. Fifteen days after the transfer to light shade. Same leaf as in Fig. 6.  $\times 260$ .
- Fig. 9. Elongation of subepidermal cells in same leaf (Fig. 6) 40 days after the transfer.  $\times 260$ .



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Fig. 10. Starch content of a sun leaf after exposure to an illumination intensity of 1000 foot-candles 16 hr. per day for each of 4 days with dry air passing over the cuticle.  $\times 260$ .

Fig. 11. Extent of palisading of a mature dark shade leaf after 80 days in the greenhouse.  $\times 260$ .

Fig. 12. Starch content of a sun leaf with the same previous experience, age and height on stem as that shown in Fig. 10 after exposure to an illumination intensity of 1000 foot-candles 16 hr. per day for each of 4 days but with near-saturated air passing over the cuticle.  $\times 260$ .

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THE EFFECT OF CUTICULAR HARDENING ON THE  
FORM OF EPIDERMAL CELLS

By R. W. WATSON

(With Plate 6 and 2 figures in the text)

Epidermal cells with radial walls showing a wavy contour have been observed in the stems, leaves and seeds of many groups of plants. In the leaves of pteridophytes such walls are almost universal (Linsbauer, 1930). In monocotyledonous leaves they are notably absent except in the Glumiflorae. Linsbauer has divided the leaves of many dicotyledons into classes according to the mode of occurrence of this character.

There has been a surprising amount of speculation with regard to the origin of this waviness. In 1821 Treviranus suggested a correlation between the wavy contours of epidermal cells and the structure of the mesophyll, and this suggestion was concurred in by Areschoug (1897), Linsbauer (1930) and Winkler (1934).

Mettenius (1865) in his work on the Hymenophyllaceae distinguished two types of epidermal cells with wavy outlines. In one the side walls were uniformly wavy throughout their height, while in the other the undulations were confined to the region adjacent to the free outer wall of the cell, so that at a lower focus the cells appeared to be polyhedral. He found that all types were straight-sided when young, and the first sign of undulation always occurred in the region of the free walls.

Askenasy (1870) working with *Ranunculus aquatilis* observed waviness in the epidermal cells of emergent leaves while this character was lacking in leaves developed under water.

Haberlandt (1882) suggested that the epidermis became crumpled through growth in a restricted space. He was chiefly interested in establishing that this crumpling increased the tensile strength of the epidermis, a theory which was controverted by Vesque in 1883.

Ambrohn (1884) confirmed the observations of Mettenius, and explained the formation of wavy contours by assuming a greater surface growth in localized parts of the radial walls. Observing that the radial walls were lightly bent from the point of their attachment to the outer wall, he postulated a resistance to waviness of growth on the part of the latter.

Differences in waviness between the cells of sun and shade leaves were noted by Areschoug (1897), and confirmed by Anheisser (1900) and others. The undulations were consistently more pronounced in the shade leaves. Numerous investigators are also agreed as to the greater tendency toward waviness on the lower side of the leaf. A few exceptions to this rule are to be found in the literature.

Brenner (1900) working with succulents produced straight-sided epidermal cells in dry air and wavy-walled cells at higher humidities. He rejected the idea that illumination directly affected the waviness.

Linsbauer (1911) in the Bromeliaceae found the original wavy contours of epidermal cells obliterated by differential secondary thickening.

Yapp (1912), Neese (1917) and Rippel (1919) reported progressive decrease in waviness proceeding from the base of the plant to the tip.

Linsbauer (1930) and Avery (1933) suggest that the waviness is due to tensions set up by differential growth rates as between epidermal and mesophyll cells.

Winkler (1934) objects to this explanation, and suggests a secretion by subepidermal cells which inhibits the growth of epidermal cell walls.

Haberlandt (1926, 1930, 1934, 1935) evolved the hypothesis that the straightness of the side walls of epidermal cells in sun leaves is due to the inhibiting effect of sunlight on genes for waviness.

While investigating the development of palisade cells in leaves on the juvenile shoots of the English ivy, *Hedera helix* L., it was noticed that the lateral walls of leaf epidermal cells in intercostal areas showed a considerable variation in waviness in response to environmental changes. The material was therefore held to be suitable for an attempt to ascertain the cause of this latter phenomenon.

All the specimens used were produced by vegetative propagation from a single original stock. The development of leaf epidermis under three sets of conditions was carefully followed. In habitat A (dark shade) the illumination amounted to about 1% of that of full summer daylight, and in habitat B (light shade) to about 10%, while habitat C was supplied with full daylight. The humidity conditions were the same in habitats A and B while the air in habitat C was on the average somewhat more humid.

The specimens were selected for uniformity, of the same age, and with approximately the same number of leaves on the stem. When grown in the above habitats such specimens follow the rule stated by Skene (1924), namely, that small leaves are produced in an illumination not far above the critical for the shade leaves, the lamina becomes largest in an intermediate light intensity, and diminishes again as light reaches the maximum represented by full summer daylight. Some details of such leaves are given in Table 1.

Table 1. *Variations in English ivy leaves under varying illumination*

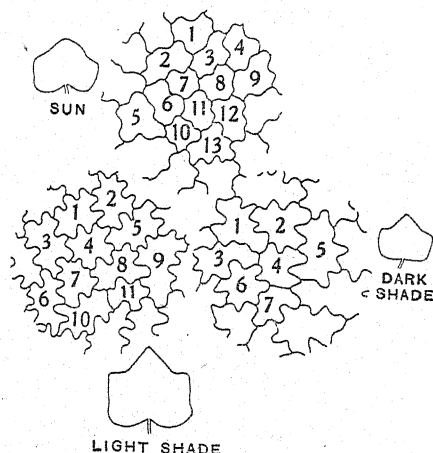
Habitat	Quantity of illumination ft. candle hr.	Area of a typical leaf sq. in.	Average cell area (unit arbitrary)	Estimated no. of upper epidermal cells per leaf	Average no. of wave crests per cell
A (dark shade)	500	1.0	1.45	300,000	8.7
B (light shade)	5,000	3.2	0.88	1,500,000	8.7
C (full daylight)	50,000	1.6	0.69	1,000,000	6.3

Text-fig. 1 shows the outlines of upper epidermal cells from a dark shade, a light shade and a sun leaf respectively, as seen by focusing on the upper edge of the middle lamella. This is the position of greatest waviness. The height of the waves as well as the number of undulations per cell is least in the sun leaf (Table 1). In light shade the depth of the waviness as well as the number of undulations per cell increases. In dark shade the cells reach their maximum size, and the number of waves per cell remains approximately the same.

To prepare transparent surface mounts of the epidermis for a study of changes in wall structure the following method was employed. A 5 mm. disk was removed from a specified position on the lamina with a circular punch. Each disk was perforated at several places with the point of a needle to allow rapid and more uniform penetration of reagents, and subjected to alternating air pressures in Carnoy's mixture for 2 or 3 min. to fill the intercellular spaces with fresh fixative. Fixation was complete in about 20 min. From 10 to 20 min. in Javel water at 90° C. followed to dissolve the cell contents and remove the

epidermis. Such treatment left the radial walls of the epidermal cells adhering to a membrane consisting of the cellulose, pectic and cuticular layers of the outer wall. These disks of cleared epidermis were washed in several changes of distilled water and subsequently stained for 24 hr. in saturated aqueous Nile blue sulphate. On removal from the stain they were rinsed for 2 min., left for 1 min. in each of three changes of dioxane and mounted directly in paraffin oil. Great care was taken with the mounts for critical observation and photomicrography. Cover-glasses not more than 0.1 mm. in thickness were selected. These were placed under light pressure until the membrane had become flat, and any excess oil had been removed. The slides were then ringed. Such mounts have retained their original brilliance of staining for three years.

In Pl. 6, fig. 1 are shown a few cells from such a surface mount of a sun leaf with the top surface uppermost, and the upper ends of the radial walls in focus. The position



Text-fig. 1. Typical leaves from habitats A (lower right), B (lower left) and C (upper), ( $\times 1/5$ ), with corresponding areas from the upper epidermis of each showing surface waviness ( $\times 140$ ).

of the middle lamella is not clear but falls somewhere within the boundary of the sinuous line. The cellulose walls lower in the cells are slightly out of focus, but can be seen to be straight. In Pl. 6, fig. 2 a photograph of the same cells at a lower focus, the lower parts of the radial walls are seen to be straight. In the adult sun leaf the wavy portion is confined to the upper parts of the radial walls of the upper epidermal cells.

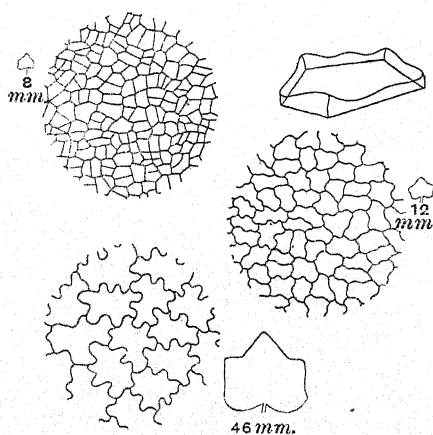
A similarly prepared surface mount of a light-shade leaf is shown in Pl. 6, fig. 3, photographed with the outer wall in focus. The course of the middle lamella is represented by the continuous dark line showing undulations of the greatest amplitude. The darker interrupted and less wavy line is the cellulose wall. The thinness of the secondary walls is partly due to the immaturity of the leaf from which this mount was made. In thin cross-sections such walls may occasionally be perpendicular but usually are more or less strongly inclined to the surface.

When a leaf is grown in an illumination not far above the critical for the shade leaf about 3 months are necessary to complete the expansion of its small lamina. Such a leaf presents a further variation in the structure of a mature upper epidermal cell. A photograph with the surface in focus is shown in Pl. 6, fig. 4. The considerably larger size of



the cell as compared with one from sun and light-shade leaves is obvious. The middle lamella may be followed as a thin rather dark line which reaches the outer boundary of the underlying cellulose wall in only a few places. In dark-shade leaves alone do the underlying secondary radial walls follow closely throughout their length the wavy outline of the middle lamella at its upper end. In mature leaves the radial walls are usually perpendicular or slightly inclined to the surface, and the waviness present at the inner ends of the radial walls is almost identical with that at the outer. To make use of a simile originated by Ambrohn (1884) the figure formed by such a wall is similar to that cut out by an inflexible rod with both ends moving over identical superimposed sine curves separated by a space equal to the height of the lumen.

If the development of upper epidermal cells in intercostal areas is followed from the time of the first expansion of the embryonic leaf, it is observed under light-shade conditions that the walls remain polyhedral until the young leaves reach a midrib length of



Text-fig. 2. Stages in the development of a light shade leaf ( $\times 1/5$ ) with corresponding stages in the development of upper epidermal cells ( $\times 140$ ). The inset shows a single cell from the 12 mm. leaf ( $\times 700$ ). Measurements refer to lengths along the midrib.

about 8 mm. Rather suddenly in the process of vacuolation the radial walls become wavy at their outer margins. Young leaves with a midrib 10 mm. long usually display incipient waviness. In Text-fig. 2 the straight-sided cells of an 8 mm. light-shade leaf are contrasted with the wavy margined cells of a 12 mm. leaf. Since, as pointed out above, the 10 mm. leaf shows incipient waviness, and since the midrib at this stage grows about 1 mm. per day in length, the waviness must appear during the course of 2 days' growth or less. If a disk from a 12 mm. leaf is cleared and stained in the manner described, an individual cell appears as shown in the inset. The outer margin of the cell has become sinuous, but the lines of junction of the radial walls with the inner tangential walls are still straight. At this stage the vertical walls are inclined only near their outer ends. The middle and lower parts of the vertical walls are straight. Only at some later time, dependent on the light intensity, do the waves extend down the vertical walls or reach the bottom of the cell and cause a corresponding waviness at the junction lines with the lower walls.

From these observations the evidence seems conclusive that the cause of the waviness in the lateral walls of upper epidermal cells in ivy leaves lies in the outer free wall. From

a careful study of this wall it should be possible to determine the origin of the waviness.

Pl. 6, fig. 5 shows a cross-section  $2\mu$  in thickness through the wavy-walled cells of a light-shade leaf, stained for several days in Smith and Nair's fat stain (Conn, 1940). The inner layer of the outer wall is anisotropic, stains blue with iodine and 70% sulphuric acid, blue with chlorzinc iodine, is completely dissolved by copper oxide ammonia, and gives a diffuse pink colour with ruthenium red. From these reactions it is evident that the inner layer is composed of cellulose with an admixture either of pectic compounds or of unsaturated fatty acids (Priestley, 1924). The outer layer which appears black stains red with Sudan III, brown with chlorzinc iodine, is left undissolved by the action of copper oxide ammonia and 70% sulphuric acid, and thus shows the microchemical properties of cuticle.

Of the two layers present in the outer wall, the inner cellulose layer and the overlying cuticle, only the latter is known to possess a sufficiently low plasticity to be capable of causing the deformities described. That the cuticle might have a share in the formation of waviness was long ago suggested by Zimmermann (1893), although he was unable to be sure whether its role was an active or a passive one. Later investigators have not accorded to his careful investigations the attention they deserve.

Pl. 6, fig. 3 is from a preparation of the outer wall of the epidermal cells of an immature light-shade leaf, separated from the leaf by immersion in Javel water at  $90^{\circ}\text{C}$ . for 20 min., stained with Nile blue sulphate and rinsed with dioxane. As shown in Pl. 6, fig. 5, the cuticle stains darkly with Nile blue sulphate but Pl. 6, fig. 3 shows a distinct differentiation between the wall in the concavities of the convolutions and that covering the rest of the cell. The lightness of staining in the sinuses is not due to an absence of fatty substance in this region. This is clearly shown by the reaction with Sudan III and other fat stains. The cuticle in these regions is, however, certainly different from the rest, since it gives up the stain when immersed in dioxane, as contrasted with the darker coloured cap which touches the middle lamellae of the radial walls of the cells only at the places where the undulations extend most deeply into the lumen.

According to those who have investigated it, the difference between the hardened cuticle and the soft fatty substances from which it develops is a question of condensation and oxidation. If we consider these changes as extending gradually over the surface of the cell, it will be evident that cell expansion will be limited at the points where the hardening first reaches the radial wall, whereas in other places the cell will still be expansible. If the darkly stained part in Pl. 6, fig. 3 represents hardened cuticle, the further stretching of the light spots to produce the waviness may be understood on the basis that here the differentiation has not proceeded far enough to limit its plasticity.

The evidence in favour of this view may be summarized as follows:

- (1) The differential staining reaction with Nile blue sulphate is evidence of some differentiation in the cuticle at this stage, and one which disappears in the mature leaf.
- (2) The lightly stained parts have expanded to form the undulations.
- (3) The waviness is produced at the period when the cuticle is hardening. In this connexion it may be stated that only after the undulations have attained considerable growth is the cuticle strong enough to be separated from the leaf and remain intact.

A consideration of the developing epidermal cell as seen in section provides additional evidence that unequal plasticity in various parts of the outer wall is responsible for the

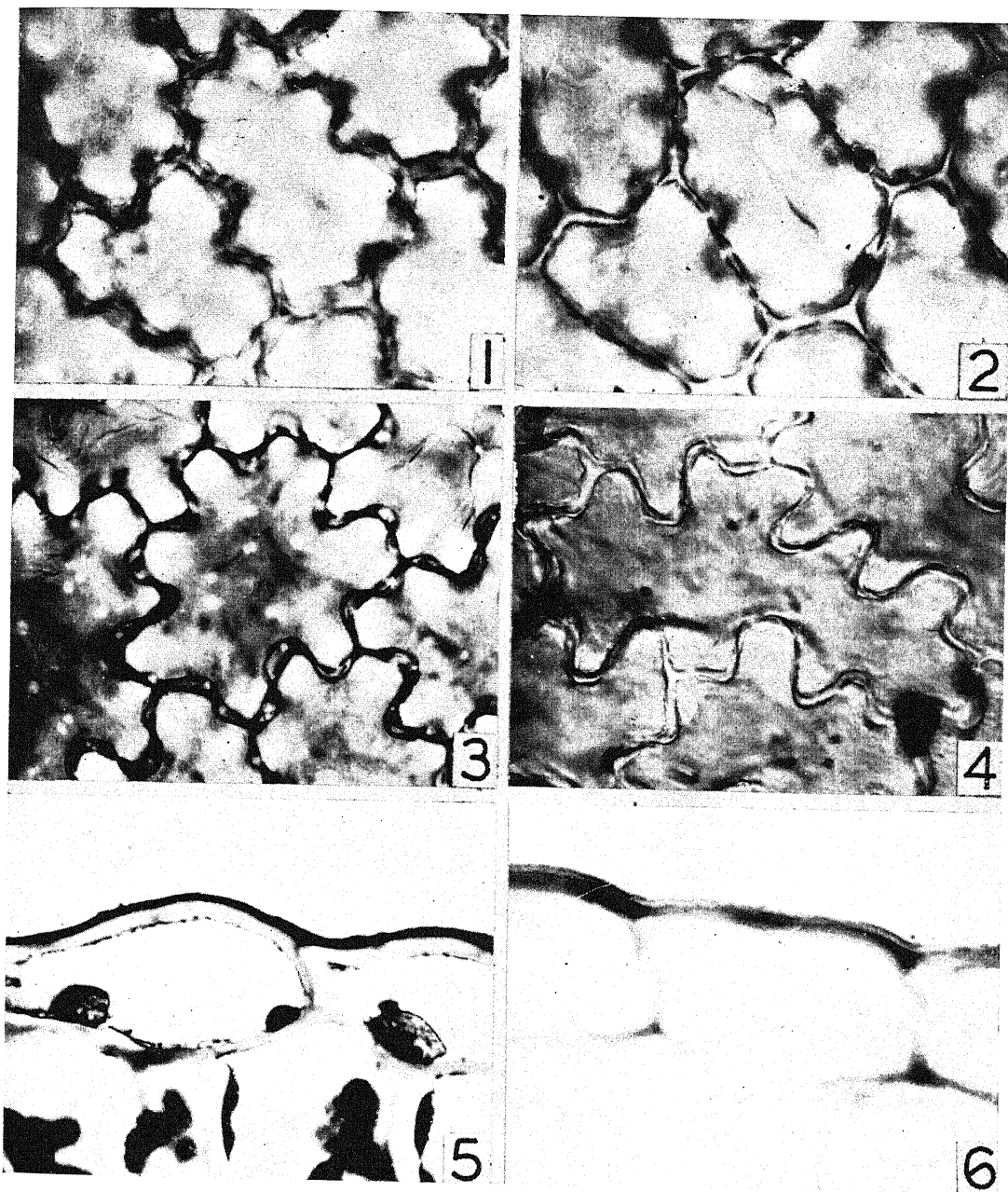
waviness. Pl. 6, fig. 6 is a section through an immature cell of a light-shade leaf passing through one of the undulations. Sections not more than  $2\mu$  thick were a necessity on account of the narrow width of the waves. This section has been treated with Javel water followed by Nile blue sulphate and dioxane as in the case of Pl. 6, fig. 3. In this case the Javel water was kept at room temperature and the treatment lasted for 4 hr. instead of 20 min. This change was necessary in order to be able to stop the process before destruction of the delicate cell walls had taken place. If the Javel water treatment is omitted, the differential staining is not obtained as may be clearly seen by comparing Pl. 6, figs. 5, 6. At the left end of the complete epidermal cell in the figure may be seen the lightly stained portion of cuticle crossing one of the convolutions, and a similar light area is visible in the cell to the right. In both cases it is clearly to be seen that the top end of the radial wall has been pushed to the left by the stretching of the outer wall in this presumably plastic spot. Such inclinations of the radial walls are the rule in developing cells of leaves grown in all three habitats and the inclination is invariably in the direction one would expect if produced by expansion of light areas such as shown in Pl. 6, fig. 3.

As the leaves reach maturity differences may be observed corresponding to the different habitats under which they were grown. In the leaves in habitat A (dark shade) while the cuticle hardens much less rapidly than in stronger light and therefore the waviness is much more pronounced, the radial and inner tangential walls of the cells remain very delicate and plastic for a still longer period, with the result that the side walls become straight but inclined to the surface and finally in many cases at least they become perpendicular so that their lower end shows a waviness identical with that at the upper end. In light shade the cell walls harden more rapidly, with the result that the waviness at their inner ends has not become so pronounced as at the outer. In the leaves of habitat C (full daylight) the cell walls rapidly become thick and hardened, and the cells are fixed in the form illustrated by Pl. 6, figs. 1, 2.

Again I should like to acknowledge my indebtedness to Dr H. B. Sifton for his invaluable and continual help, to Dr D. H. Hamly who aided unsparingly in the photomicrography, and to Miss C. B. Ross for her assistance in many ways.

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WATSON—THE EFFECT OF CUTICULAR HARDENING ON THE  
FORM OF EPIDERMAL CELLS





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#### EXPLANATION OF PLATE 6

All the figures represent upper epidermal cells from intercostal areas of leaves of the English ivy, *Hedera helix* L.

Fig. 1. Cells from a sun leaf with the outer tangential wall in focus.  $\times 750$ . The waviness of the walls at this height is quite evident.

Fig. 2. Same cells as in fig. 1 with the inner tangential wall in focus.  $\times 750$ . At this level the radial walls are straight.

Fig. 3. Cells from an immature light-shade leaf showing differential staining of the outer tangential wall.  $\times 770$ . The lightly stained areas have stretched to produce convolutions.

Fig. 4. Single complete cell from a leaf grown in dark shade.  $\times 750$ . In this leaf the waviness of the radial walls extends throughout their height.

Fig. 5. Cross-section through an undulation in the radial wall of an immature light-shade leaf.  $\times 1200$ .

Fig. 6. Cross-section similar to the one in fig. 5, stained after treatment with Javel water.  $\times 2200$ . Note how the radial walls have been bent by the stretching of the lightly stained portions of the outer tangential wall.

# THE ENERGY CHANGES ASSOCIATED WITH PLANT RESPIRATION

By K. WOHL AND W. O. JAMES

*Department of Botany, Oxford*

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## INTRODUCTION

It has been realized for a long time that the respiration of plants and animals is accompanied by transformations of energy, and much has been said about the utility of these changes to the organism. Respiration, like any other process which goes on spontaneously, involves a decrease of free energy; and, since plant life is always accompanied by respiration, it is likely that some use is made of the decrease. This does not necessarily mean that the energy is used to do work in the restricted sense given to the word in thermodynamics, i.e. to do mechanical or electrical work upon the environment. It is one of our main objects to explain the meaning we attach to the phrase 'utilization of energy by plants'.

In order to keep respiration going there must be a continuous supply of free energy. This is normally obtained by plants from the sun. In photosynthesis they transform the radiant energy of sunlight into chemical energy by converting carbon dioxide and water into oxygen and sugar or, more exactly, into some activated intermediate of photosynthesis which relapses spontaneously into sugars. Our title excludes these stages and we shall not consider further how the plant acquires chemical energy, but what is done with it when acquired. We thus make mentally a convenient, though quite unreal, separation between photosynthesis and other plant activities.

The bulk of the sunlight absorbed by plants is never effective by means of its free energy, but is degraded into heat and is used in transpiration to evaporate water from the cell surfaces of the mesophyll. According to the data of Brown & Escombe (1905) for *Polygonum Weyrichii*, the energy absorbed in transpiration may exceed that transformed

in photosynthesis by more than 30 to 1. In transpiration actual mechanical work is done since water is lifted from the soil to leaf-level. This work may even be regarded as useful to the plant since the water of the transpiration stream and the soil nutrients it contains are needed by the leaves. The amount of work done is, however, very small. Even in raising the water to the top of a tall tree 60 m. high it would only be equivalent to 0.024 % of the heat of vaporization of the same amount of water. To adhere to the strict mechanical definition of work is clearly not the way to render impressive the work done by the sun through the agency of plants. Moreover, the work of transpiration is almost entirely independent of respiration, resulting directly from the absorption of sun energy by an entirely physical process. No chemical reactions are involved.

There is probably no definition of respiration which is satisfactory for all purposes; in the present context we may regard respiration as the formation of carbon dioxide, or other highly oxidized end-products of metabolism, by spontaneous breakdown of organic substrates, which, on account of the decrease in free energy involved in the reactions, is able to drive the metabolic processes and other vital activities. At a later stage (pp. 241 et seq.) we shall be able to describe more fully what is implied by the word 'drive'.

The oxidation of inorganic materials by chemosynthetic bacteria is an excluded, though somewhat similar, process. It is possible that such oxidations are linked strictly to sugar synthesis and co-exist with a true respiration. They are thus functionally analogous with photosynthesis rather than respiration though chemically very different.

It is justifiable to speak of the utilization of energy by plants in two respects: first, in the building up processes of growth, and secondly in maintenance. In the active growth phase, part of the sugar which disappears is consumed to carbon dioxide and another part is built up into more permanent plant constituents such as cellulose and proteins, or temporarily into reserves like starch and fat. All these substances are rich in chemical energy, but it can hardly be said that their energy has been utilized in any way by the plant. Cellulose formation, for example, is accompanied only by a very slight increase in free energy; the glucose units incorporated into its molecule still exist without serious change in the final product. None of their chemical energy has been utilized during their condensation but is entirely locked up in the cellulose, which the average plant has no means of decomposing. It is the mechanical properties of cellulose, not its imprisoned chemical energy, which makes it important to plants. They are rather in the position of a man forced to build his house with blocks of coal instead of putting them on the fire for the sake of their heat. Applying the same analogy to starch, the coal is merely being stored. The chemical energy may be utilized later when starch has been reconverted to sugar, but it has not been utilized merely by putting the coal into the cellar. The situation is rather similar in protein formation.

A fully grown organism in which active increase has stopped is not an inert block; it is a system out of equilibrium with its environment and consequently suffering spontaneous changes that are inevitable. Nevertheless, it maintains itself in a more or less steady state, which can only be done with a continuous supply of free energy. This is secured by the continuous breakdown of carbohydrate, but when we attempt to decide what part of the energy transformed has done anything towards maintaining the organism and what part of it has been 'wasted' we meet with great difficulties. As in any ideal steady state, the only material change observable is the disappearance of the initial reactants (starch or other reserves) and the appearance of final reaction products (carbon



dioxide and water): the entire loss of energy appears as heat, which is transferred to the environment. No work is done and no alteration is brought about within the organism; yet it cannot be said that the energy has been 100% wasted, since without respiration the plant would die.

What, therefore, is meant by saying that a plant utilizes energy, and what part of the energy of the sugar decomposed is actually made use of? The meaning of such questions is clear when they concern the performance of external work, such as lifting a weight. But the amount of external work done by a plant at any stage is small, and negligible when compared with the energy output of respiration.\* The amount of mechanical and electrical work done inside a complex plant in cell division, protoplasmic streaming, bio-electric currents, etc. is also extremely small. The free energy of the sugar decomposed is actually utilized almost entirely in bringing about chemical changes, as was implied in our definition of respiration on p. 231, i.e. in the syntheses of active growth and in the metabolic changes which persist in the adult. We shall see that the energy concerned in these processes may fairly be said to be utilized, and this is not the less true because in the adult phase all the energy eventually escapes from the plant as heat. Before this happens it has undergone intermediate transformations entailing important material consequences within the living tissues.

#### HEAT PRODUCTION

Before turning to the chemical utilization of respiratory energy it may be useful to get a clear idea about the utility or non-utility of the heat produced.

The escape of heat from rapidly respiring material, such as germinating seeds and opening flower-buds, is very familiar. It is commonly demonstrated by the rise of temperature when the respiring organs are surrounded by more or less efficient lagging, usually a Dewar or thermos flask. Under favourable conditions such rises of temperature may be considerable; e.g. 20° C. by germinating peas, equivalent to 34.6 Cal. per g. dry weight (Pierce, 1912). The heating of moist grain in store results from a relatively fast respiration. With water contents between 15 and 17%, the CO<sub>2</sub> output of wheat is more than trebled for each 1% rise (Bailey & Gurjar, 1918). Wheat with 18% moisture showed a rise of 2.6°, and other grains much more (Gilman & Barron, 1930). A high external temperature increases the heat production by accelerating respiration rates and, at the same time, reducing the rate of loss to the surroundings. The rise of temperature is thus accelerated. The temperature on a malting floor with barley germinating in a thick layer may rise several degrees Centigrade.

If grass, or any other plant material, is stacked while still alive, a rapid rise of temperature results from its own respiration. We have observed a temperature of 38° C. in a heap of grass cuttings within 18 hr. of mowing at a time when the air temperature was 15.5° C. Spontaneous heat production may eventually lead to the firing of hayricks built when the grass was still slightly moist. The respiration of bacteria, especially of the *Bacillus coli* type, soon becomes active in the warm mass and raises the temperature to about 42° C. Above this temperature *B. caletactor* survives until the temperature reaches about 70° C. The hay then becomes sterile, but abiotic oxidations, very slow at normal temperatures, become appreciable at this point. If the stack is small, the loss

\* 'Consider the lilies of the field, how they grow; they toil not...'.

from its surface balances their heat production, but in a large rick the temperature inside continues to rise until ignition-point is reached. There is a limiting bulk/surface ratio below which ignition will not occur however damp the hay (Haldane & Makgill, 1923).

We have spoken of heat as the form of energy finally escaping from plants, implying that their living tissues are unable to transform heat into any other form of energy. Living tissues are clearly not heat engines able to use a temperature difference for transforming part of the heat by either a continuous or periodic process into mechanical work. The inability of cells to transform heat into work has been demonstrated in great detail with muscle fibres, which have been selected for the experiment on account of their exceptionally high energy turnover (Gray, 1931). Corresponding demonstrations with plant material are out of the question because of the smallness and ill-defined nature of the work done.

In transpiration a small part of the heat involved is doing work at constant temperature, and with the trifling exception of osmotic work (see p. 248), this is the only way in which heat can be shown to perform work in plants. Moreover, this heat is taken as such mainly from the environment. If there is any special source of heat which increases the temperature, transpiration is accelerated and the heat responsible for the rise of temperature must provide for the increased loss of heat to the environment as well as for the cooling effect of the additional transpiration. Respiration may be regarded as a special source of heat making a very small contribution to transpiration.

Apart from this very minor point, the effect of heat is better considered not from the standpoint of energy but from that of temperature. A rise of temperature accelerates the rates of chemical reactions. In living tissues a moderate rise of temperature, up to about 35° C., is usually favourable. The supply of heat necessary to maintain a temperature higher than that of the surroundings depends entirely on circumstances. One can be certain that it is necessary to supply heat continuously from a special source only if the accelerated reactions are endothermic. This is the case with transpiration, but not with respiration or the sum total of metabolism. If the temperature is kept constant, it does not affect the energy balance at all.

Doyer (1915) has shown that the production of heat is itself increased by a rise of temperature up to 35° C.

Table 1. *Heat production by wheat seedlings in Cal. per hr. per kg. original weight (data of Doyer)*

20°	25°	30°	35°	40°
2.601	3.428	6.034	7.450	6.268

A rise of temperature is turned to various accounts in the bodies of warm-blooded animals. On account of their diffuse form, their lack of any efficient heat lagging and the cooling effect of transpiration, the higher plants do not show any corresponding effect. Only rarely, as during the opening of large inflorescences and, to a less extent, during the development of massive fruits, does any natural rise of temperature occur which may be ascribed to respiration.

The developing inflorescences of *Arum maculatum* show a rise of several degrees within the spathe (Church, 1908). The heat production is localized in the naked upper part of the spadix and, after wrapping this about with cotton-wool, a rise of 13° C. has been observed. By the evening of the day of opening the temperature had fallen again.

In the first stages of flowering the terminal club of the spadix is glutted with starch, and this is almost wholly consumed by the evening. Even at the time of maximum heat production, about 5 p.m., there is a readily detected loss of starch. There can thus be little doubt that the heat and temperature rise result from starch consumption in respiration. Church speculated that the warmer temperature within the spathe might have biological significance by attracting insects in the cool weather of early spring when the inflorescence develops. *A. italicum* shows the same phenomenon even more strikingly; its spadices may reach a temperature considerably over blood heat and become perceptibly warm to the touch. Church observed the same simultaneous depletion of starch from the heat-producing tip of the spadix as in *A. maculatum*. Ripening apples are also said to maintain themselves at a few degrees above the atmospheric temperature.

Artificial and post-mortem phenomena, such as malting and the heating of damp hay, are without interest in this connexion, and in general it may be said that a plant is fairly closely adjusted to the temperature of its environment. With strong illumination and conditions which retard transpiration, there may be a considerable rise of temperature in leaves. This is not due to respiration but to the absorption of light and to the slowness of its loss as heat. It is thus clear that even the indirect effects of heat production in respiration are of very minor importance to plants.

Experiments have been made in the past to decide how much of the energy decrease associated with respiration appears as heat. The decrease of total energy is given by the decrease of the heat of combustion of the tissue during the period considered, and this is a measurable quantity. The energy lost by the tissue can appear either as heat or as work done outside the tissue. In either case it can no longer appear as heat when the tissue is afterwards burnt. Since external work done by plant tissues is insignificant, and may not occur at all under experimental conditions, it must be expected that the decrease of energy measured will have appeared as heat during the period of respiration to the extent of practically 100%. This will occur in the growth phase just as much as in the adult. The values obtained experimentally are actually close to 100%, and the small deviations recorded may well be attributed to the errors inherent in the difficult technique. Rodewald (1887, 1889) estimated the heat production as 93% of the heat of combustion of the sugar consumed in kohlrabi stems, and as 99.2% in apples. Algera's (1932) figures for the whole period of a culture of *Aspergillus niger* were 3299/3518 Cal. = 94%.

A very different result was obtained by Doyer (1915) who worked on the heat production of germinating wheat. The quantities she measured were the amount of heat given out to a stream of air at a controlled temperature; the heat of combustion of wheat samples at successive stages; and the quantity of CO<sub>2</sub> given off. The differences between successive heats of combustion were taken as a measure of the loss of total energy by the seedlings. Assuming that the CO<sub>2</sub> all originated from a complete oxidation of starch, and that its heat of formation was 2.56 Cal. per g., she was able to calculate the heat equivalent of the CO<sub>2</sub> given off during each period of measurement. The actual heat production could thus be compared with the loss of total energy as calculated (*a*) from the heat of combustion of the whole grain, and (*b*) from the production of CO<sub>2</sub>. An error is introduced into the calculation by the fact that in the earliest stages there is some utilization of fats as well as starch; and Malhotra (1933) has shown that the calorific value of air-dried wheat is not constant but sinks from 3.492 Cal. per g. to 3.301 after 8 days of germination.

This error is not great enough to obscure the main results. It is unfortunate that all the determinations were not carried out at a single temperature. At 20° C. the percentage loss of energy appearing as heat varies from 38 to 95. The lower values could only be explained by means of work done by the plant. It is true that in these experiments some work was done by the seedlings, viz. the work of raising water in guttation. Guttation is characteristic of young seedlings in an atmosphere of low evaporating power and the work is similar to that done in transpiration. Suppose 1 kg. young wheat seedlings to consist of 20,000 plants averaging 5 cm. high (an extreme case), and that it raises 10 mg. water per hour. The work of guttation would then be  $20,000 \times 5 \times 0.01 = 1000$  cm. g., corresponding to 0.024 Cal., while the heat of combustion unaccounted for is 707-4631 Cal. We are therefore compelled to conclude that Doyer's results at 20° C. are erroneous.

Table 2. *Energy losses of germinating wheat seedlings in Cal. per hr. per kg. initial weight (data of Doyer)*

Day	Exp. 1 at 20° C.		Exp. 2 at 25° C.	
	Heat emitted	Loss of total energy Cal. from heats of combustion	Heat emitted	Loss of total energy Cal. from CO <sub>2</sub> given off
2	—	83	363	2,135
3	710	1417	540	3,802
4	2143	2250	2938	6,277
5	2790	3833	3216	6,886
6	—	4000	4341	8,837
7	2869	7500	—	10,068
8	—	—	—	10,854

According to the experiment at 25° C. (Table 2) the heat emitted is always less than half the heat of formation of CO<sub>2</sub>, calculated on the assumption that the CO<sub>2</sub> is entirely produced by complete combustion of starch. If this result is taken at its face value, we must suppose that a large part of the CO<sub>2</sub> had actually been produced by processes with a smaller heat of reaction, such as fermentation. As the figures obtained at 20° C. indicate an impossible result, those at 25° C. do not give one confidence in making such an unlikely and otherwise unconfirmed assumption.

#### UTILIZATION OF ENERGY IN GROWTH AND MAINTENANCE

(1) *Growth.* During growth sugars are converted into substances of greater energy content, the additional energy being derived from the breakdown to CO<sub>2</sub> of some part of the sugar available. Not all the energy decrease of the lost sugars passes into the new substances, and it is difficult to determine the proportion that is thus used. At first sight it appears possible to obtain an estimate by comparing the CO<sub>2</sub> output during a given period with the decrease in heat of combustion. This would give an estimate of the total—not free—energy utilized and could only be applied in the absence of simultaneous photosynthesis. Doyer (see above) measured the two quantities required in darkened wheat seedlings, but unfortunately in separate experiments under different conditions. Molliard (1922) made the comparison directly with moulds.

In the absence of all other reactions than the complete oxidation of sugar or starch to CO<sub>2</sub>, the decrease in heat of combustion of the plant and its substrate,  $\Delta$ , would be equal to the heat of formation of the CO<sub>2</sub> given off,  $H$ . If part of the energy of CO<sub>2</sub>



formation is utilized in the production of substances like fats and proteins with a high energy content, the heat of combustion should decrease less and  $\Delta$  become less than  $H$ . The difference  $H - \Delta$  would thus be a measure of the utilization of respiratory energy and could be expressed as the percentage  $100(H - \Delta)/H$ . There is, however, another possibility, viz. the existence of incomplete oxidations yielding energy but no  $\text{CO}_2$ . This is known to occur in seedlings forming carbohydrates from fats and in succulents forming malic acid from sugars. In such cases  $\Delta$  exceeds  $H$  and the method would lead to the absurdity of a negative result. If the amount of incomplete oxidation is small relative to the amount of respiration as a whole, there will be errors less easy to detect. Molliard found that his estimates of  $H$  and  $\Delta$  were equal so that the 'efficiency of respiration' was nil. He concluded that no respiratory energy was chemically utilized by the moulds, but it seems more likely that the result was due to a fortuitous compensation, within the limits of experimental error, of energy utilization and incomplete oxidation. The growth of the moulds would consist very largely in the formation of cell-wall materials with an energy content only very slightly greater than the sugar of the medium from which they were formed.

(2) *Maintenance*. Only the living cell can build up and maintain the large content of energy which protoplasm represents. But this does not mean that the energy must be readily released at death. Meyerhof (1924) performed the experiment of suddenly killing a large number of respiring erythrocytes in a calorimeter by adding acrolein. No measurable heat was given off. He further showed that in eggs narcotized with 0.01 % phenylurethane, oxygen consumption and heat production were unchanged, but cell division was stopped. No extra heat appeared due to a saving of energy by the stoppage of division. This, however, is not surprising. The act of killing may only involve some slight specific change of structure or colloidal condition in a protoplasmic constituent, e.g. the breakdown of some barrier which separated active chemical agents from one another in the living state. The change of energy associated with a process of this kind would be very small indeed, and well within the experimental error of even the most accurate calorimetry.

Such separations maintaining systems out of equilibrium both with the environment and with one another are familiar in biology. Oxidases, for example, exist in cells side by side with oxygen from the environment and oxidizable pigments formed within; but only act upon them fully when life is destroyed. The continuous maintenance of potentials implied by such disequilibria might result from resistances associated with spatial separation or, alternatively, maintenance may only be achieved by the continuous expenditure of energy.\*

It is a facile assumption that the energy change revealed by the continuous liberation of heat from mature tissues which are neither doing external work nor synthesizing appreciably, is a measure of the energy of maintenance. The assumption will not stand closer inspection, and we are driven to the view that what is revealed may equally well be a mere energy wastage. To determine the efficiency of respiration in maintaining the steady state, we should need to know the rate of spontaneous breakdown of all substances other than respiratory substrates, together with their different states of aggregation, structure and concentration. We should also need to know how much sugar was consumed in making a unit replacement and to compare the sugar requirement this indicated with

\* For a fuller discussion see James (1932*a, b*).

the total sugar lost by respiration. We are, however, very far from possessing such complete knowledge as this on which to base a quantitative calculation.

The most obvious energy wastage is found among primitive and especially among anaerobic saprophytes. Yeast under nitrogen will continue to release energy from sugar by vigorous fermentation when growth is at a standstill and, so far as can be seen, energy utilization is virtually nil. The action seems to be the result of uncontrolled enzyme activity on susceptible materials, and is not at all graduated to the metabolic needs of the organism (cf. Stevenson, 1930). Alcohol is far from being the only product of alcoholic fermentation. Butyric acid production occurs widely in bogs and other anaerobic places, but it also does not occur as a pure butyric acid fermentation; very variable quantities of alcohol, lactic acid and acetic acid are formed at the same time. The direction of the yeast or bacterial metabolism is largely at the mercy of circumstance. Starting from such a condition, evolution may be envisaged as a progressive control of expenditure of material and energy. The breakdown of sugars has become canalized into a more or less restricted path of slow oxidation, preventing the formation of the multitudinous partially oxidized end-products found in bacterial metabolism. Restriction in the amount of breakdown has also accompanied restriction in kind, as shown in Table 3, but this is not to say that the breakdown which does occur in the higher plant is efficiently used. There are here no data to set a limit to speculation.

Table 3

	$Q_{CO_2}$	
Yeast	60-100	Meyerhof, 1925
<i>Aspergillus niger</i> , 2-day culture	78.0	Kostychev, 1927
4-day culture	11.5	
<i>Ribes nigrum</i> leaf buds	2.0	Garreau, 1851
Barley 7-day seedlings	1.6	James, A. L., 1938
Sunflower, germinating seeds	1.5	Kidd, West & Briggs, 1921
3-month-old plants	0.2	

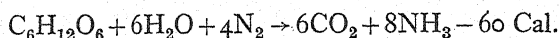
$Q_{CO_2}$  = cu.mm.  $CO_2$  per mg. dry weight per hour. Data recalculated where necessary.

#### UTILIZATION OF ENERGY IN CHEMICAL REACTIONS

The preceding section has shown that a quantitative treatment of the 'utilization of free energy' is not possible for the physiology of a plant taken as a whole. There are too many uncertainties, including possibilities of incomplete oxidations and the production of intermediate substances with energy levels both higher as well as lower than those of sugar. A quantitative treatment can only be carried out if a definite process is known by which free energy is released and another process by which part of this free energy is used. Since the energy turn-over of plants is mainly connected with the chemical processes of metabolism, the utilization of free energy by chemical reactions must be considered as well as their production of it. This necessitates a few words concerning the general principles of chemical energetics.

The reactions of metabolism may be divided, like others, into those which are spontaneous and those which are not possible without assistance from some second reaction or other source of energy. Chemists and biologists alike need an indication whether any given reaction is spontaneously possible or not. This information may very often be obtained from the heat of reaction. If the heat of reaction is very great—as it

is in the reaction  $C + O_2 \rightarrow CO_2 + 94.5 \text{ Cal.}$ —we can be sure that the reaction can only happen in the direction shown by the arrow, in which it is exothermic. This is always true except at extremely high temperatures with which biological reactions have no concern. The opposite, endothermic reaction is in this case excluded, but in reactions with only a moderate heat of reaction this is not always so. We may take as an example the assimilation of nitrogen with the breakdown of glucose by *Azotobacter*, which may be schematically represented thus:



In spite of the negative heat of reaction this can and does take place. The same may be said of any chemical equilibrium which may, in theory at least, be approached from either side. Of the two processes concerned, one is necessarily exothermic and the other endothermic, but both occur spontaneously. It is, therefore, evident that the heat of reaction does not determine the direction of reaction and is not a true measure of affinity. The heat of reaction is the decrease of total energy in a system as a result of the reaction, but affinity is measured by the decrease of free energy. Free energy differs from total energy on account of the random movements of the atoms characteristic of temperature. The decrease of free energy (= affinity) differs, therefore, from the decrease of total energy (= heat of reaction) whenever the random movements change during the course of the reaction. At absolute zero, temperature movements being nil, free energy and total energy are identical, and so also would be the affinity and heat of reaction of any chemical change able to occur. At biological temperatures the energy of random atomic movement is often much smaller than the heat of reaction, and any changes of random movement produced by the reaction are usually of minor importance. Whenever this is so, the heat of reaction gives a good approximation to the affinity, and the reaction can only occur spontaneously in the direction in which it is exothermic (Berthelot's rule). But the higher the temperature rises the less certainly can the affinity and direction of reaction be predicted from the heat of reaction alone. At room temperature Berthelot's rule holds under practically all conditions, if not less than 15 Cal. are developed per mol. produced by the reaction. With a heat of reaction as large as this equilibrium lies at an almost complete formation of the products of the exothermic reaction. Berthelot's rule very often holds with smaller heats of reaction and fails above our numerical limit only when giant molecules are involved in which numerous linkages participate in the reaction, as, for example, in the denaturation of proteins.

At any given temperature the difference between heat of reaction and affinity increases with increasing difference in the type of molecular movement before and after the reaction. Such changes are particularly great when the state of aggregation is changed, and especially if a gas is produced or consumed. The natural tendency of molecules and atoms is to move independently and to fill the space presented to them uniformly with their irregular movements. They can only be prevented from doing this by energy in the form of special attractive forces holding them together. In other words, there is always a natural tendency to form a gas, and this tendency becomes effective whenever the attractive forces of liquefaction or solidification are not great enough to overcome it. These forces of attraction may contribute very largely to the heat of reaction if the reaction includes a change of state, and the formation of a gas, which involves the breaking of bonds, is usually endothermic for this reason. Nevertheless the affinity (= decrease of

free energy in the reaction) may still remain positive, and so the endothermic gas formation may take place spontaneously as a result.

In reactions involving numerous linkages the heat of the chemical change may be positive in spite of gas formation, and then the affinity is still greater. The greater the number of gas molecules that are formed in the reaction, the more it is favoured in the direction of their formation. Similarly, the formation of two gas molecules from a single gas molecule is favoured on account of the increased independence of movement of the constituent atoms. Thus we arrive at a very simple qualitative rule. In a reaction in which no gases are formed, there will not be any great difference between heat of reaction (= decrease of total energy) and affinity (= decrease of free energy). The affinity is greater than the heat of reaction in any reaction which causes an increase in the number of gas molecules; and, in a range of reactions, the difference increases with increasing gas formation. The formation of solutions is not usually associated with such large differences between heat of reaction and affinity, because the solvent cannot be regarded as an empty space, being already overcrowded with solvent molecules. Further complications are also introduced by ionization and solvation.

There is one further factor not mentioned in the preceding paragraphs which needs to be pointed out. The heat of reaction between solids or liquids on the one hand and gases on the other is practically independent of the gas pressure, since even at high pressures the mean distance between gas molecules is much larger than the range of the molecular forces of attraction. Similarly, if solutions are involved, the heat of reaction is more or less independent of the concentration except when it is very high. The decrease of free energy (affinity), on the other hand, is closely related to the concentration of the reacting gas or dissolved substance. Hence all that has been said above applies strictly to a given set of standard conditions. These are taken as 1 atm. partial pressure for a gas and a *N* solution for a dissolved substance.

We have already pointed out that the natural tendency of molecules to spread out and fill any space presented to them is able to overcome the forces of attraction; and this becomes more apparent as the space in which they are able to evade one another is enlarged. Hence the affinity of the formation of a gas or dissolved substance becomes greater as the concentration falls, and dilution or reduction of pressure favours solution and gas formation. The effect of dilution on affinity is, however, relatively small when we are considering reactions with a large energy turn-over. For every gaseous or dissolved molecule formed, affinity increases at room temperature by 1.36 Cal. for a tenfold dilution.\* Conversely, a tenfold dilution decreases the affinity of gas consumption by a like amount.

We are now in a position to consider the energy relations of those reactions which are supposed to occur in respiration. A number of relevant data are collected in Table 4. They are calculated for 25° C. but can be used as a good approximation at all biological temperatures. Reaction 1 in this table is usually regarded as the overall reaction of aerobic respiration. For purposes of calculation the hexose is assumed to be glucose, but the inclusion of fructose would not involve any serious alterations. In this reaction the number of gas molecules is the same, viz. 6, on both sides of the equation. Hence, with solid glucose, pure water, and gas pressures of 1 atm., there is little difference between

\* The factor 1.36 is strictly valid only for 25° C., but being proportional to the absolute temperature does not change very much within the biological temperature range.



the heat of reaction (decrease of total energy) and affinity (decrease of free energy). These values are shown in Table 4 as  $H$  and  $A$  in columns 1 and 2 respectively. The further columns of the table show the values of  $H$  and  $A$  under a variety of conditions. Columns 3 and 4 show the effect of bringing glucose and  $\text{CO}_2$  into solution. There is a considerable difference in the heat of the reaction because the heat of solution is great, but the change in the affinity value is very small and need not be discussed. On the other hand, the effect of dilution on the heat of reaction is so small as to be negligible, but its effect on the affinity value is shown in columns 5 and 6. Column 6 is calculated for conditions which may be taken to represent a normal biological state, viz. oxygen and

Table 4. *Energy relations under varying conditions*

Temperature 25° C.

	Pure substances. $\text{O}_2$ , $\text{CO}_2$ and $\text{H}_2$ at 1 atm.		Normal solutions*, except $\text{O}_2$ and $\text{H}_2$ at 1 atm.		$\text{O}_2$ and $\text{CO}_2$ at air con- ditions†	'Bio- logical' con- ditions‡
	1	2	3	4	5	6
	$H$	$A$	$H_1$	$A_1$	$A_2$	$A_3$
(1) $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$	674.0	690.4	713.5	677.3	712.5	709.8
(2) $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2$	21.3	59.3	40.8	52.8	66.4	71.9
(3) $\text{C}_2\text{H}_5\text{OH} + 3\text{O}_2 \rightarrow 2\text{CO}_2 + 3\text{H}_2\text{O}$	326.4	315.6	336.3	312.3	323.1	319.0
$2\text{C}_2\text{H}_5\text{OH} + 6\text{O}_2 \rightarrow 4\text{CO}_2 + 6\text{H}_2\text{O}$	652.7	631.1	672.7	624.6	646.1	648.0
(4) $\text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \rightarrow$ $2\text{CH}_3\text{CHO} + 2\text{CO}_2 + 2\text{H}_2\text{O}$	124.9	153.9	~139.8	~148.9	~161.6	~169.8
(5) $\text{CH}_3\text{CHO} + \frac{5}{2}\text{O}_2 \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O}$	274.6	268.3	~286.9	~264.3	~275.5	~270.0
$2\text{CH}_3\text{CHO} + 5\text{O}_2 \rightarrow 4\text{CO}_2 + 4\text{H}_2\text{O}$	549.1	536.5	~573.7	~528.5	~551.0	~540.1
(6) $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow$ $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2$	15.8	65.5	~ 30.5	~ 60.8	~ 74.4	~ 85.3

$H$  and  $H_1$ =heats or reaction in Cal. per mol.  $A$ ,  $A_1$ ,  $A_2$  and  $A_3$ =affinities (decreases of free energy) in Cal. per mol.

\* As data for dissolved acetaldehyde and butyric acid are lacking, the values for the free energy of both substances in the liquid state have been used for the 1*N* solutions. This affects the figures in the last four columns only slightly.

† Other conditions as in the preceding column.

‡  $\text{O}_2$  and  $\text{CO}_2$  at air conditions;  $N/100$  glucose,  $N/1000$  alcohol and butyric acid,  $N/10,000$  acetaldehyde;  $\text{H}_2$  at 0.01 % of 1 atm. The biological partial pressure of  $\text{H}_2$  is very uncertain.

$\text{CO}_2$  at normal air conditions§ and glucose in  $N/100$  solution. The hexose concentration in the sap of barley leaves is of about this order. In column 5 only oxygen and  $\text{CO}_2$  have been adjusted to biological conditions, and it will be seen at once that any variation of sugar concentration possible in living cells will have only a small effect on the affinity value.

Heats of reaction, as ordinarily given in physico-chemical tables, apply to the standard conditions used in our column 1. Such figures are often quoted in connexion with the energy of respiration (reaction 1), so it is of interest to see how they compare with our adjusted estimate of the free energy of the process in column 6. This shows an excess of 35.8 Cal. over column 1, but in a reaction with such a high energy turn-over as 709.8 Cal., this is of little importance. It may, therefore, be said that, in practice, the heat of combustion of glucose gives a reasonably good approximate, though not strictly accurate,

§ If the concentration of dissolved  $\text{CO}_2$  is such that an equilibrium is established with gaseous  $\text{CO}_2$  of any pressure the free energy of  $\text{CO}_2$  is the same in both states, so that the same affinity values are obtained whether  $\text{CO}_2$  reacts as gas or as dissolved substance. Thus air conditions mean 0.03 atm. or  $10^{-5}/N$  solution.

value of the energy that may become 'available' in respiration and no serious errors are likely to have resulted from its use.

In reaction 2 of Table 4 the position is very different. This reaction is of interest as being the summation of alcoholic fermentation in yeast and at least an important factor in the anaerobic respiration of higher plants. The standard heat of reaction is here much smaller than in reaction 1, but as there is a formation during the reaction of 2 gas molecules, the affinity exceeds it and the difference is still further increased by the transition to biological conditions. During the course of fermentation the concentration of alcohol may increase to the order of a *N* solution; but it will be seen from column 5 that this involves little reduction of the affinity value. The chief interest here attaches to the fact that the free energy decrease of the reaction is 3.4 times greater than the heat of reaction as usually quoted, and that a correspondingly greater usefulness would appear possible. It is, however, as well to remember that the adjusted value is still only 10% of the corresponding value in the aerobic oxidation, reaction 1, and that other factors discussed more fully in the final section must be taken into account in assessing the biological utility of this reaction.

Reaction 3 is the combustion of alcohol. The values for the combustion of two molecules of alcohol, added to the values of their formation (reaction 2), give the values for the complete combustion of sugar (reaction 1). Reaction 4 is the partial oxidation of sugar with formation of  $\text{CO}_2$  and acetaldehyde. This reaction is usually considered to occur in the early stages of both fermentation and aerobic respiration and to provide a point of departure for many syntheses. Reaction 5 is related to reactions 4 and 1 as reaction 3 is related to reactions 2 and 1. Reaction 6 is the butyric acid fermentation already mentioned as occurring in anaerobic plants.

In reaction 4—and to an even greater extent in reaction 6—the number of gas molecules is increased so that the standard affinity *A* exceeds the standard heat of reaction *H*. The values for butyric acid formation are surprisingly similar to those for alcoholic fermentation. The heat of reaction for the formation of butyric acid is still smaller, the affinity still larger under every condition considered in the table. The 'biological' value in column 6 exceeds the standard heat of reaction in column 1 by the factor 5.4.

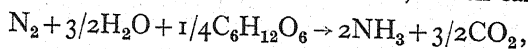
For many biological reactions free-energy data are not available. In these cases the heat of reaction must be used as an indication of the direction in which the reaction will proceed spontaneously as has always been done in the past. If the heat of reaction is considerably smaller than 15 Cal. per mol. produced (cf. p. 238), it is advisable as a further indication to use the rules given on p. 239 for standard conditions and the special conditions under which the reaction takes place.

#### DRIVEN REACTIONS—SYNTHESIS

The decomposition of sugars whether aerobically to  $\text{CO}_2$  and water or anaerobically to  $\text{CO}_2$  and alcohol proceeds in a number of stages with very different energy relations. Not all of them are spontaneous, and we may suppose that those requiring an energy supply are driven by those of high affinity. Precisely the same is true of all the syntheses occurring in the plant, since these, if taken by themselves, would imply an increase of free energy. They must be related to those stages of respiration that occur with a sufficient decrease, and this necessity also implies some chemical mechanism for the connexion.

Connexion between two chemical processes is brought about by one or more molecules common to both of them, but the particular molecules linking biological reactions are usually still unknown.

(1) *Nitrogen assimilation.* The transformation of  $N_2$  to  $2NH_3$ , a reaction probably carried out by nitrogen-fixing bacteria, may be given as a first example. The reaction  $N_2 + 3H_2O \rightarrow 2NH_3 + 3/2O_2$  is impossible by itself because it would involve a free-energy increase of 161.9 Cal. If the oxygen produced is given an opportunity to oxidize glucose, the free energy falls by 192.2 Cal. and the total reaction, which can be written

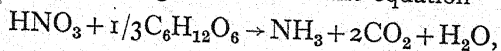


is accompanied by a free-energy decrease of 10.3 Cal. The overall reaction is thus driven by the free-energy decrease of its oxidation stage, in spite of its negative heat of reaction of -15 Cal., as shown on p. 238. Putting it in another way, we might say that the enormous tendency for the formation of  $CO_2$  is utilized to form a product of high energy content, viz.  $NH_3$ . The energetic efficiency of the linkage can be said to be

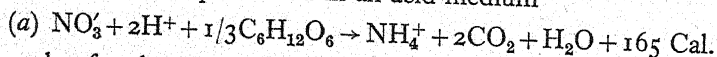
$$161.9/172.2 \times 100 = 94.0\%.$$

It is, however, necessary to point out that the subdivision on paper of a total reaction into a driving and a driven one has the character of an interpretation and can sometimes be carried out theoretically in more than one way.

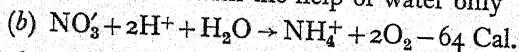
(2) *Nitrate reduction.* Energy changes are involved in at least four stages of protein synthesis: absorption, reduction, amination and condensation. Absorption is not considered here (but see p. 249). The energy relations of nitrate reduction by *Chlorella* have been studied by Warburg & Negelein (1920) in some detail. The reaction is endothermic and is associated in the absence of light with sugar consumption. If the rate of absorption of nitrate by the cells is increased artificially, respiration becomes partly 'anaerobic' with excess nitrate replacing free oxygen as oxygen supply. This was shown both by an altered gas exchange and by a greatly increased sensitivity to cyanide poisoning. After an initial period in which ammonia is used in protein synthesis, 2 mol.  $CO_2$  come off for each mol.  $NH_3$  formed, in agreement with the equation



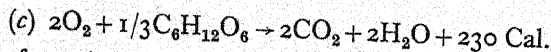
or, since the experiment was performed in an acid medium



The positive value for the standard affinity shows that the reaction can proceed spontaneously. It can also be subdivided into a driven and a driving reaction. The transformation of nitrate into ammonia with the help of water only



cannot occur because of the negative affinity. The oxygen formed is used, however, to oxidize carbohydrate



The affinity of reaction (c) is thus amply sufficient to drive reaction (b), so bringing about the complete reaction (a). The energetic efficiency of the linkage is here

$$64/230 \times 100 = 28\%.$$

Nothing is known of the detailed chemical mechanism by which reactions (b) and (c) are linked.

(3) *Carbohydrate condensation.* The energy requirements of amination and amino-acid condensation are very small compared with those of nitrate reduction. The same is also true of sugar condensation to form higher carbohydrates, as will be seen from Table 5. The heat of formation of sucrose from glucose and fructose is particularly small and falls near the limit of experimental accuracy. If we may judge from the affinity of the transformation of glucose to sucrose, the only figures available, the affinity values must

Table 5A

	Glucose→ fructose	Glucose→ $\frac{1}{2}$ sucrose	Glucose + fructose→ sucrose	Glucose→ starch ( $C_6H_{10}O_5$ )	Glucose→ cellulose ( $C_6H_{10}O_5$ )
Heat of formation	-2.1	-1.6	-1.1	-3.7	-4.2
Affinity of formation	—	-1.45	—	—	—

Table 5B

	Glucose	Fructose	Sucrose
Heat of solvation	-2.4	-2.2	-1.0
Solubility in mol. per litre	3.45	6.19	2.64
Affinity of the reaction solid→solution (uncorrected)	+0.73	+1.08	+0.58
Heat of transformation $\alpha$ sugar→ $\beta$ sugar	-0.166	-1.24	—
Affinity of transformation $\alpha$ sugar→ $\beta$ sugar	-0.4	—	—

All figures are Cal. per mol.

all be very similar to the heats of reaction. The heats of formation of the polysaccharides are only slightly greater than that of sucrose, and this is why a good deal of synthesis may result in plants with the expenditure of very little energy. The oxidation of 1 mol. of glucose liberates enough energy to condense more than 100 mol. to cellulose, assuming a high efficiency. The actual efficiency of this and similar reactions is not yet known. During the first and second days of germination of barley, the relations between 'hexose' respired and 'hexose' condensed to cellulose, hemicellulose and maltose were as follows:

*Data from James & James (1940)*

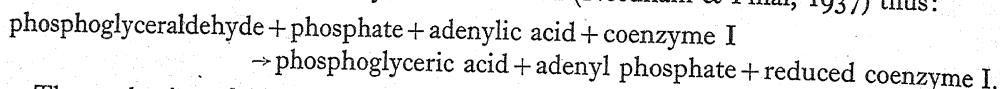
	Hexose respired as calc. from CO <sub>2</sub> emitted mg.	Hexose condensed to cellulose, hemicellulose and maltose mg.	Ratio
1st day	23.0	8.6	0.27
2nd day	77.0	19.3	0.25

i.e. approximately 4 mol. of hexose were respired for every one condensed.

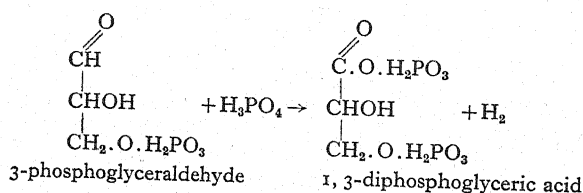
(4) *Phosphorylation.* Phosphates play a catalytic role in a number of biological reactions, and the study of their action has provided us with one of the most fully worked out examples of reaction-linkage yet known. The synthesis of the phosphate carrier adenylyl pyrophosphate has come to be regarded as of first class biological importance. It involves an increase of free energy probably in the neighbourhood of 10 Cal. per mol. and has a measured heat of reaction of 11 Cal. (Meyerhof, 1930). The energy is available biologically through coupling with a specific oxidation (triosephosphate→phosphoglycerate) without which phosphorylation will not occur. Conversely the oxidation does not take place in the absence of phosphate.



The substance oxidized is triosephosphate, and unphosphorylated glyceraldehyde or dihydroxyacetone will not do. The substance reduced is the pyridine nucleotide coenzyme I. The total reaction may be summarized (Needham & Pillai, 1937) thus:

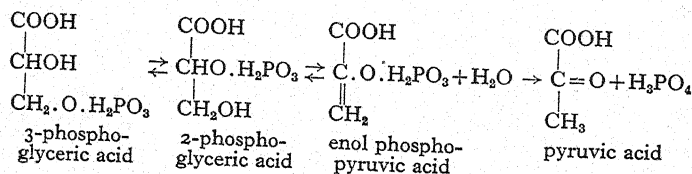


The mechanism of this reaction, or, in other words, the method of linking its oxidation of phosphoglyceraldehyde with its phosphorylation of adenylic acid, has been worked out by Warburg & Christian (1939) and by Negelein & Brömel (1939) using muscle preparations as sources of the necessary enzymes. The primary oxidation product of phosphoglyceraldehyde was identified by Negelein & Brömel as 1, 3-diphosphoglyceric acid in which the additional phosphate is linked with the aldehyde group, oxidizing it to carboxyl in the process



the  $\text{H}_2$  being accepted by coenzyme I. The 1, 3-diphosphoglyceric acid easily loses the extra phosphate in the no. 1 position which is transferred to adenylic acid (or adenylyl phosphate), in the presence of a suitable enzyme, to give adenylyl phosphate (or adenylyl pyrophosphate).\*

The phosphate in the no. 3 position is also transferred to adenylic acid in a further series of reactions:



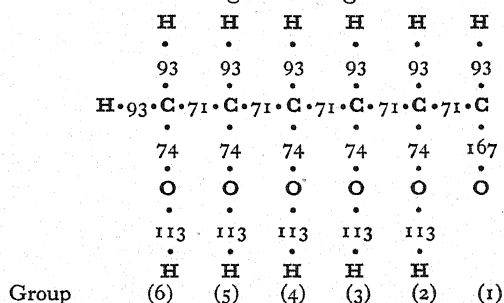
The hydrolysis of the last stage has a decrease of free energy = 11.25 Cal. (Lipmann, 1941) which is about equal to the increase of free energy in the accompanying synthesis of adenylyl pyrophosphate from phosphate and adenylic acid (see above).

By means of these reactions the energy of the unstable carboxyl-phosphate and carbonyl-phosphate linkages is stored in the adenylyl pyrophosphate and so made available for further synthesis. Hydrolysis of the pyrophosphate again releases ~10 Cal. per mol.

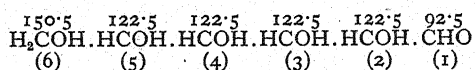
(5) *Formation of acetaldehyde.* The conception of a driving and a driven reaction can also be applied to the formation of alcohol and similar compounds such as acetic, lactic and butyric acids from sugar. These have relatively high free energy contents and could not be formed without the driving process of  $\text{CO}_2$  formation. In their case it is a trifle more difficult to translate the idea of driving and driven processes into numerical terms, because we are here dealing with a rearrangement of atoms within the sugar molecule. We may say in a general way that the O-atoms concentrate on certain C-atoms to form  $\text{CO}_2$ . This process has such a high affinity (i.e. proceeds with so great a loss of

\* Two methods of naming are in common use for these compounds. Adenylic acid = adenosine monophosphate; adenylyl phosphate = adenosine diphosphate; adenylyl pyrophosphate = adenosine triphosphate.

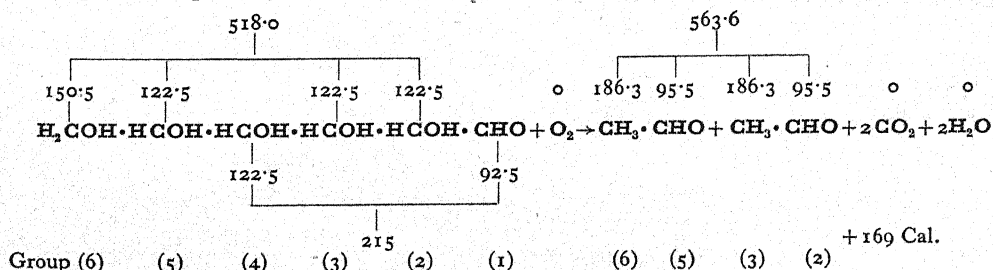
free energy) that it occurs even though the grouping round the remaining C-atoms becomes less likely than it was before (i.e. increases its free energy). To make this conception quantitative we must introduce figures for the energy of the linkages between atoms and decide which linkages are broken and what new linkages are made. To do this it is easiest to operate with total energies, not with free energies, and to neglect the state of aggregation of the molecules or, rather, to assume that they are free molecules in the gaseous state. These simplifications do not affect the main issue and could be corrected for if they did. We take the partial oxidation of sugar with formation of  $\text{CO}_2$  and acetaldehyde as an example. This reaction is usually considered to occur in the early stages of both fermentation and aerobic respiration and to provide a point of departure for many syntheses. The energy relations of the whole reaction are summarized in Table 3, reaction 4. For the further analysis we can write the glucose molecule in the following form, convenient for showing the energetic value of every linkage:



The heat of combustion of 'gaseous glucose' corresponding with these values can be found by imagining that the molecule is first separated into single atoms and that these are individually oxidized into  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . The result is 733 Cal. We may distribute this heat of combustion among the groups formed by the separate C-atoms with their attendant H- and O-atoms, if we divide the 71 Cal. shown for the C—C linkage equally between its two C-atoms. We then arrive at the following values for the heats of combustion of the six groups.



These figures give a measure of the energy-level of each group since they give the maximum energy which it can produce in the presence of oxygen.  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , the most stable substances into which they may be converted, have a zero value. The formation of acetaldehyde may now be described as a splitting of the glucose molecule between groups 3 and 4, followed by a complete oxidation of groups 1 and 4 to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Groups 2+3 and 5+6 form 2 mol. of acetaldehyde. This may be represented



The heat of combustion of the two groups 1 and 4 which are completely oxidized is shown to be 215 Cal., but only 169 Cal. appear as heat of reaction. The remaining 46 Cal. have gone to increase the energy level of the other part of the glucose molecule, so that the heat of combustion of the 2 aldehyde molecules is 563.6 Cal., whereas the heat of combustion of the corresponding groups while still in the glucose molecule was only 518 Cal. This is true even though the 2 aldehyde molecules contain 1 less H-atom than the glucose groups 2, 3, 5, and 6. We can thus say that the complete oxidation of groups 1 and 4 supplies chemical energy with an efficiency of  $100 \times 46/215 = 21\%$ . The spontaneous production of molecules with an increased content of free and total energy could be similarly treated in other cases.

#### REVERSIBILITY

There is also a qualitative aspect of energy utilization for chemical purposes which is of biological importance. Respiration is subdivided into numerous chemical steps by which a variety of intermediates and by-products are formed. These afford as many opportunities for the coupling of respiration to synthetic processes. The decrease of free energy in any single step is necessarily small when compared with the decrease caused by complete oxidation. The smaller each decrease can be made the greater becomes the number of chemical events that can be produced by respiration. We might, therefore, say that this minute subdivision of free energy, each portion associated with a special reaction, is the means by which the plant succeeds in using the free energy of respiration for all its needs.

A most elegant example is afforded by the action of phosphate on the oxidation of phosphoglyceraldehyde (see p. 244). In this reaction phosphate has taken the part often allotted to water in the oxidation of a carbonyl group. The oxidation with water is virtually irreversible and proceeds with a large decrease of free energy to the formation of a stable acid group. The oxidation with phosphate, on the other hand, is reversible with a relatively small  $\Delta F$ , the unstable carboxy-phosphate product being still labile and able to transfer phosphate and energy to suitable acceptors like adenylic acid instead of scattering them as unattached phosphate and heat (see also Kalckar, 1942).

The division into small energy decrements has also another important consequence. A process occurring in solution, or involving a solution or gas, does not go to completion if its free-energy decrease is small. The free energy of the partners in the reaction depends on their concentration and the reaction will proceed only until the concentrations of the reactants and their products have changed so that the free energy is equal on both sides of the chemical equation; in other words, until it comes to an equilibrium. In consequence a series of chemical equilibria are set up in plant respiration which may be shifted to one side or the other by the intrusion of very small amounts of free energy. Shifting equilibria of this sort are generally supposed to play a considerable part in metabolism. Thus it has been found possible to reverse the oxidation of triosephosphate described on p. 244 and to reduce phosphoglycerate by decomposition of adenylyl pyrophosphate (Meyerhof, Ohlmeyer & Mohle, 1938). The chemical reversibility referred to here applies only to single steps; the reversal of the complete reaction, carbohydrate to  $\text{CO}_2$ , is photosynthesis and requires large doses of the sun's free energy.

## WORK

There are no tissues in plants capable of doing external work on a scale to compare with muscles. The slow displacement of soil particles by the growth of thick roots is an analogous but not very impressive phenomenon. The work done in lifting weights during growth and by tropistic curvatures is also very small. The bulk of a tree's canopy is not lifted from ground-level by the expenditure of respiratory energy (as is sometimes implied), but the dry matter is synthesized from atmospheric  $\text{CO}_2$  in situ, and the water content raised by transpiration.

The term 'internal work' is sometimes convenient but is ambiguous and needs to be clearly defined. There are numerous conditions in the plant of which it can be said that their free energy is in one way or another above the lowest level to which it might relapse by some spontaneous process given suitable conditions. The spontaneous process may or may not involve interaction with the plant's environment. Products with a high content of chemical energy may be able to change to those with a lower; highly dispersed structures, on account of their large amount of surface, may represent a higher energy level than more compact forms. Concentrated solutions may be reduced to a lower energy level by dilution, and substances are held apart that may reduce their free energy by mixing. Van't Hoff has shown that in theory all these higher states may be attained from the lower by the performance of mechanical work such as the falling of a weight, and that if the process is carried out reversibly the increase of free energy is equal to the mechanical work done. If we give the name 'work' to everything that could be produced by mechanical work in such ways, then we might speak of chemical work, the work of forming structure, concentrating a solution and so on. In reality, however, the internal structures of plants such as lignified or cellulose walls, protoplasmic membranes, plastids and even the dispersed colloidal state, originate during the actual chemical process of their formation. The same is often true of concentrated solutions. It cannot be said that any work has been done specifically to create the structure, only that the decrease of free energy connected with the chemical process is not as great as it would have been had the substances been formed with their lowest possible content of free energy, i.e. as crystals, dilute solutions, and so on. Even if a dilute solution is afterwards concentrated, it is not done by mechanical work as will be shown later (pp. 248 et seq.). To avoid confusion, therefore, we prefer not to use the word 'work' in this connexion ('internal work', 'chemical work', etc.) and to confine it to the strict thermodynamic use of mechanical work and electrical work as discussed below.

The differences of free energy associated with different concentrations (cf. p. 239) and states of aggregation are usually much smaller than the changes of free energy in chemical reactions. It may be remarked in passing that the transition from a compact to a dispersed state is not always accompanied by an increase of free energy. Where molecules with hydrophile groups are concerned, the formation of a colloidal sol or gel from the compact state may be a spontaneous process accompanied by decrease of free energy. The biological significance of colloids lies, to a large extent, in their capacity for adsorption on account of their high specific surface; or, in other words, for determining the distribution and arrangement of adsorbable substances. The amount of energy involved in all this is very small when compared with the total energy turn-over of the living system.

A further difficulty arises if mechanical effects inside the plant are treated as work.



In thermodynamics work is the energy exchanged between the system and its environment, which alters the energy of some mechanical or electrical arrangement belonging to the environment. If other kinds of energy were inserted into the conventional thermodynamic equations as work, they would lead to erroneous conclusions. This difficulty could be avoided by a suitable definition in each case of the system and its environment. When a cell grows against a tissue pressure it could be regarded as the system doing work on an environment of other cells. Even the cell wall being stretched by the swelling of its contents might be considered outside the system. Cell division, on the other hand, furnishes an example of visible mechanical effects even more intimately included in the cell. The amount of energy involved appears, however, to be negligible and fails to affect the total energy consumption measurably (Gray, 1931).

A somewhat different consideration applies to the kinetic phenomena of protoplasmic streaming or the active movements of chromosomes in living cells which have been likened to eels in a box (Strangeways, 1922). Movement in cells is retarded by friction and has to be maintained by a continuous supply of free energy, which is eventually converted into heat of friction. The immediate source of free energy being within the cell, a state characterized by internal movements cannot maintain a constant free energy value and so presents serious difficulties to a thermodynamic treatment. These need not be considered here, as kinetic phenomena are of only minor importance in plants.

The decrease of free energy associated with the dilution of solutions is not commonly made to do work. It is apparent in the tendency of concentrated solutions in contact with water to dilute spontaneously by diffusion until the most probable state, having the lowest free energy, (*viz.* homogeneity), is reached. If the solution is separated from water by a semipermeable membrane, dilution can take place as a reversible process in which the decrease of free energy is almost entirely used for doing work. This maximal amount of work is done if the solution expands against a pressure practically equal to the osmotic pressure of the solution. Under any other conditions less work is done. The decrease of free energy associated with the mixing of two separately dissolved substances is hardly ever used to do *mechanical* work. As pointed out above, a cell may be regarded as doing work when it expands as a result of its osmotic pressure against the turgor pressure of its surrounding cells. On the other hand, if a relatively rigid cell wall grows so fast that a negative pressure develops in the cell, *i.e.* if its suction pressure exceeds its osmotic pressure, the walls do work by overcoming the pressure difference in their slow movement. This has been observed in young tissues (Bennet-Clark, Greenwood & Barker, 1936; Bennet-Clark & Bexon, 1940). The amount of energy involved is very small, as in the other processes mentioned above. The source of energy in this case is the production of relatively concentrated solutions by metabolic processes, and only in this sense is it dependent on respiration (*cf.* section on salt accumulation, p. 249). To make this clear it may be useful to say a little more about the energetics of osmosis and diffusion.

The decrease of free energy,  $\Delta F$ , often has the same order of magnitude as the decrease of total energy,  $\Delta U$ , in chemical reactions and mechanical processes with solid bodies. The distinction between free and total energy may, therefore, come to be regarded as a detailed refinement. This idea would be utterly misleading if applied to gases and dilute solutions. When a dissolved substance diffuses irreversibly into water, its free energy falls, but the total energy remains unaltered and no energy is exchanged with the

environment outside the fluids. If the dilution is carried out reversibly, in a thermostat in the presence of a semipermeable membrane, i.e. by letting the volume of the solution increase against an external pressure equal to the osmotic pressure, the total energy is not altered either, since the initial and final states are the same as in the irreversible process mentioned above. But work has been done equivalent to the decrease of free energy in the system at the expense of heat absorbed by it from the thermostat. The free energy is thus not determined by the total energy of the system but by the probability of distribution of the dissolved molecules, all distributions being iso-energetic. In general, total energy and distribution together determine the free energy and only when distribution remains unaltered is total energy the deciding factor.

The heat of the environment is made available to do work in the osmometer in the following way. As long as the temperature is kept constant the dissolved molecules have a free translational energy of  $\frac{3}{2}kT$ , equally distributed among the three dimensions of space. This energy of random movement produces the osmotic pressure and the dissolved molecules do osmotic work at the expense of its component  $\frac{1}{2}kT$  allotted to the direction in which the external pressure is overcome. The energy deficit thus caused is immediately made good by collisions with water molecules, which in turn draw energy from the environment by further impacts. Heat is thus transferred spontaneously from the environment and no other source of energy comes into play. Conversely, if work is done upon the system as in a tissue subjected to mechanical pressure, the energy of the dissolved molecules is increased and then dissipated as heat to the water and so to the environment. If the action is slow, it is quantitatively reversible. Molecules freshly formed by chemical reaction, or newly brought into solution, also spontaneously acquire the appropriate heat energy by collision, and it is again the environment which provides the energy that enables them to do osmotic work.

Electrical energy can theoretically be transformed completely into mechanical energy and vice versa and the two are thermodynamically equivalent. Electromotive forces are present in animal tissues and to some extent in plant tissues also. They afford an example of the increase of free energy due to segregation. Ions of different kinds or concentrations may migrate apart and produce an electric field instead of diffusing irreversibly or reacting directly with one another. Such bio-electric currents may help to bring about chemical results such as polarization and to degrade further energy to heat.

#### SALT ACCUMULATION

It would appear from recent investigations that an increase of free energy is connected with the accumulation of soil solutes by roots. It is now a commonplace of absorption studies that nutrient ions may be accumulated within suitable cells and tissues to a concentration greatly in excess of the external. Since free energy increases with concentration, the question arises: What is the source of this energy and what machinery applies it?

Two kinds of salt accumulation have to be distinguished: the secondary accumulation of ions in mature tissues, and the more effective primary salt accumulation of rapidly growing tissues which are in the meristematic or juvenile phases or which have become active by rejuvenation (Steward, 1935). In secondary ion accumulation only one ion, usually the cation, increases its concentration while the ions of other sign (anions) decrease and the product of the two concentrations remains constant. The cation con-

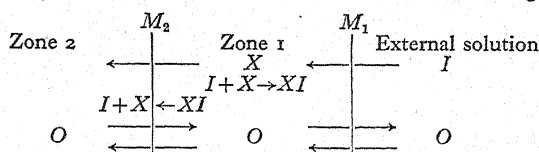
centration may increase threefold. It is possible that this result is an expression of the Donnan equilibrium. For this to apply it has to be assumed that the membrane is impermeable to an anion occurring in the cell sap, or that an acid like  $\text{H}_2\text{CO}_3$  is continuously produced, so that it maintains a higher concentration inside the cell than out in spite of its ability to penetrate the membrane. Because a macroscopic separation of electric charges is energetically impossible, a cation such as  $\text{K}^+$  can only enter the cell either together with  $\text{Cl}'$  (or another anion) or in exchange for a cation leaving the cell simultaneously (cf. Redfern, 1922; Stiles, 1924). The escaping cation may be  $\text{H}^+$ , derived from respiratory  $\text{H}_2\text{CO}_3$ . Either entering  $\text{Cl}'$  or escaping  $\text{H}^+$  migrate according to the normal diffusion gradient from a relatively concentrated solution to a more dilute one, i.e. in a direction in which free energy is decreased. Thus their movements are able to play the role of driving process for the accumulation of  $\text{K}^+$  ions so that the migration of all the ions concerned is associated with a net increase of free energy, as in any other spontaneous process. The final state reached—the Donnan equilibrium—is the state of lowest free energy possible so long as the cell membrane remains semipermeable and  $\text{CO}_2$  is being produced. If either of these conditions is abolished, a further decrease of free energy can take place as the concentrations of all the ions become equal inside the cell and out. The accumulation of cations by this secondary process is linked with the respiratory stream of free energy only in so far as it results in the production of  $\text{H}_2\text{CO}_3$  or anions unable to penetrate the cell membranes.

Primary salt accumulation, as the name implies, indicates an accumulation of the total salt, or more exactly an increase of the ionic product, since more of one ion may be taken up than of its associate. The product may increase more than 40-fold. This presents a much more complex problem than the secondary ion accumulation described above. Briggs (1930) has suggested a mechanism of primary salt accumulation which is equivalent to a modification of the mechanism of ion accumulation given above. In this hypothesis the diffusion of  $\text{K}^+$  and  $\text{Cl}'$  ions inwards against their concentration gradients is coupled with the diffusion of  $\text{H}^+$  and  $\text{HCO}_3'$  outwards with their gradients. In order that this may go on with a decrease of free energy it is necessary to suppose that the membrane has phases of permeability towards cations only, alternating with phases of permeability towards anions only. Up to the present there is no direct evidence that such membranes are ever formed by cells, nor has any theory been developed to show how they might arise. Further, it seems clear that salt accumulation is not so closely connected with  $\text{CO}_2$  formation as this theory would require, since salt accumulation occurs only in rapidly growing cells and depends on free oxygen and on a sufficient concentration of sugar to a higher degree than can be accounted for by their effect on  $\text{CO}_2$  production. Though this does not necessarily rule out the theory altogether, but may only mean that it tells but half the story, a mechanism more intimately connected with metabolism—and not merely with one of its end-products—does seem more likely. Not only salts but non-ionizing substances such as the sucrose in sugar-beet accumulate against a concentration gradient and, in the more thoroughly investigated actions of the kidney, transfer of various substances against their concentration gradient has been observed.

In the absence of a membrane with alternating permeability, free energy must be supplied from some source other than the diffusing substances themselves. The amount of free energy needed is very small and from the standpoint of economy of energy Briggs's mechanism has little advantage over one which does not couple salt accumulation solely

with diffusion of other ions, but rather with spontaneous chemical reactions, like the processes discussed in previous sections. The principle of a method by which a chemical reaction could be coupled with salt accumulation is given below.

Beside the external solutions from which the salt is being absorbed, it is necessary to suppose the existence of two membranes and two reaction zones arranged as shown below:



Zone 1 is separated from the external solution by membrane 1 and from zone 2 by membrane 2. The ions,  $I$  and  $O$ , of a strong electrolyte penetrate the membrane  $M_1$  into the reaction zone (zone 1) where a substance  $X$  is being continuously produced.  $X$  is capable of combining with the ion  $I$  to form the compound  $XI$ . Neither  $X$  nor  $XI$  can penetrate membrane 1 into the external solution, but both may pass membrane 2 into the second zone. In this zone, either in its general volume or at its boundary surface,  $X$  is consumed whether it is in the free form or combined as  $XI$ . The released  $I$  ions are incapable of penetrating membrane 2 and accumulate indefinitely. The ions  $O$ , of opposite charge, migrate into zone 2 to maintain electro-neutrality.

Such a scheme as this can be applied to plant tissues in more than one way. We may take as an example a young tissue which is rapidly accumulating salts and whose cells are vacuolating. Membrane 1 may then be supposed to represent the plasma membrane at the outer surface of the cytoplasm; zone 1 the cytoplasm itself; zone 2 the developing vacuole and membrane 2 the tonoplast between vacuole and cytoplasm. According to our suggestion the ion  $I$  accumulates in the growing vacuoles because it combines in the cytoplasm with some intermediate product of metabolism— $X$ . This substance has so high an affinity for the material of the inner membrane that it is able to penetrate it to the surface of the vacuole, even when combined with the ion  $I$ . Here, at the interface between cytoplasm and vacuole,  $X$  may suffer its next transformation to  $X'$ , which is sufficiently drastic to release  $I$ . The substance  $X'$  remains capable of diffusing back into the cytoplasm through the tonoplast; but  $I$  is unable to do so and must accumulate in the vacuole.  $X'$  may be irreversibly destroyed and replaced by a new  $X$  molecule in the normal course of metabolism, or it may be retransformed in the cytoplasm to  $X$  by coupling with processes of sugar-breakdown, so that a very small quantity of  $X$  would be sufficient to cause the accumulation of  $I$ . It is most probable that  $O_2$  is required for these processes, if only for the reason that  $O_2$  is necessary for nearly all the useful activities of the plant (cf. next section) and especially for the chemical transformations of  $X$  and its derivatives. The assumption that the cytoplasm-vacuole interface is a catalytic surface is not at all improbable because the vacuole begins as a collection of points at which freely soluble substances are developed. These link together and enlarge during the growth of the cell merely owing to the osmotic properties of the system called into being.

The ion  $O$ , of opposite charge to  $I$  and able to pass through both membranes, is held within the vacuole by its electrostatic attraction to  $I$ . It is not necessary to suppose that it will occur in equivalent amount to  $I$ , as in the external solution, since ionic exchanges as described under secondary ion accumulation may occur both in the cytoplasm and



in the vacuole itself with ions derived either from respiratory carbonic acid or other sources. Indeed it frequently happens that one ion of an absorbed salt accumulates to a markedly greater extent than the other, even in young and rapidly absorbing tissues (Steward, 1932*a*). We venture to suggest that this mechanism fits the known facts of primary salt accumulation as described on p. 250 and involves no assumptions unfamiliar in cytology.

#### BIOLOGICAL VALUE OF THE ENERGY OF ANAEROBIC RESPIRATION

Pasteur suggested that fermentation provided energy enabling yeast to grow to some extent in the absence of oxygen. Pfeffer (1900) applied the idea to the anaerobic respiration of higher plants, and Oparin (1938) has extended it so far as to suppose anaerobiosis the precursor of an oxidative metabolism in evolution. According to Table 1, the free energy of the fermentation reaction is 9-10% of the free energy of the hexose oxidation, and there is evidence that sugar decomposition is accelerated under nitrogen.

Assuming that the aerobic and anaerobic reactions approximate to reactions 1 and 2 of Table 4, the ratio of sugar breakdown in nitrogen and air is about 3 in barley (James & Hora, 1940) and 3.9→4.9 in apples (Blackman, 1928). Hence, from the data of Table 4 (last column), it follows that the relative rates of free energy release are:

Aerobic	708.3
Anaerobic (barley)	211.2
Anaerobic (apples)	316.8

These anaerobic figures are far from negligible in comparison with the aerobic, nor are the known uncertainties of the data great enough to be likely to make them so (cf. Fidler, 1933). Nevertheless, the conception of anaerobic respiration as a provider of energy can only be true to a very limited degree. Though it must be true of anaerobes, it cannot apply at all to some other types of cells, such as blood cells (Meyerhof, 1924) and to strict aerobes among the bacteria. Very young seedlings do not show the increased sugar consumption under nitrogen shown by older tissues; indeed, they very soon cease to show any activity at all (Leach, 1936; James & Hora, 1940).

Yeast itself is unable to multiply under nitrogen beyond a concentration of about  $28 \times 10^4$  cells per cu.mm., although fermentation continues briskly. If the culture already contains this concentration when brought into nitrogen, no divisions occur (Brown, 1892).

The value of anaerobic respiration among the higher plants is very doubtful. Although they may survive for a time under nitrogen and afterwards show no lasting injury when brought back into air, they are far from being able to perform their natural functions during their nitrogen experience. Functions which may involve the performance of appreciable work, such as tropistic curvatures against resistance, are not the only ones affected. It has been shown that, although cell division can require only a minute expenditure of energy, it will not occur anaerobically (Gray, 1931). Translocation of solutes is also inhibited in the higher plants by rigorous exclusion of oxygen (Mason & Phillis, 1936). Germination comes to an abrupt stop under nitrogen or in the presence of traces of  $H_2S$ , an oxidase poison (James & Hora, 1940). In rapidly growing tissues there is a rough parallelism between salt absorption and respiration-rate at low oxygen pressures (Steward, 1935; Steward, Berry & Broyer, 1936). Although rapid absorption of ions is associated with a consumption of starch, as in regenerating potato tissue (Steward, 1932*b*) or of sugars as in excised barley roots (Hoagland & Broyer, 1936), this

is not in itself enough. There must also be an adequate supply of oxygen. Steward (1935) noted that absorption fell off more rapidly than respiration in percentages of oxygen below atmospheric. In the total absence of oxygen there was actual loss of solute from the tissues. We have ourselves noted a similar effect with a dye. Methylene blue is rapidly absorbed by barley roots from a dilute and aerated solution but passes out again if the roots are laid on a moist filter-paper and enclosed in an exhausted Thunberg tube; though it is retained if the filter-paper is merely moistened with aerated water. On readmission of oxygen the blue colour, temporarily bleached by the reducing power of the roots, reappears on the filter-paper in contact with their external surfaces. The movement of substances against their normal diffusion gradient has also been shown to be associated with increased oxygen consumption in kidney. Absorption of water by potato disks is almost suppressed by the absence of oxygen and  $10^{-6}$  heterauxin simultaneously increases their water absorption, respiration and loss of starch (Reinders, 1938).

Since water absorption depends largely on transpiration, the influence of respiration on it may rather be catalytic, as by the production of a substance increasing permeability or reducing wall pressure, than by supplying free energy. It is possible that in one or other of these ways respiration has a contribution to make towards positive sap pressure, though it seems unlikely to be a very large one.

It has long been known that leaves floated on sugar solutions will only synthesize starch from them if well aerated. Recently, McCready & Hassid (1941) have shown that barley shoots infiltrated with 5% glucose will only form sucrose if oxygen is present. There was no gain of sucrose after 24 hours in nitrogen, a large gain in oxygen and an intermediate one in air. The very low energy requirements of these syntheses could certainly be covered by anaerobic glycolysis.

Streaming of the protoplasm is one of the most sensitive of all vital activities to a shortage of oxygen. Besides the direct observation of the necessity for free oxygen, there is also its reaction to narcotics which at very low concentrations accelerate its rate and at higher concentrations retard it (Ewart, 1903). This is similar to the effect of chloroform on the oxygen consumption and  $\text{CO}_2$  emission of respiration (Thoday, 1913; Irving, 1911). Very dilute doses of cyanide inhibit respiration (James & Hora, 1940; Thomas & Fidler, 1941), and also retard protoplasmic streaming. It is worthy of note that the inhibiting doses of cyanide and chloroform (provided the cells do not contain abundant polyphenols) depress the absorption of oxygen more than the emission of  $\text{CO}_2$ . The cyanide doses are also known to inhibit the action of plant oxidases and peroxidases (James & Cragg, 1941). The significant point is thus made that it is oxygen consumption, not sugar degradation, that is the essential factor. Further information may be derived from the use of indole-3-acetic acid (heteroauxin). This accelerates oxidative respiration rates at a concentration of  $10^{-6}$  (Reinders, 1938), and under suitable conditions it has been shown to accelerate protoplasmic streaming at  $10^{-8}$  (Sweeney & Thimann, 1938). The suitable conditions include a simultaneous supply of sugar and adequate oxygen. It is noteworthy that at  $10^{-8}$ , although the effect on streaming can be observed, the effect on respiration eludes direct measurement. The same authors have made the very significant observation that streaming has to compete with other processes for the available oxygen. If their consumption is increased—as by an overdose of indole-3-acetic acid or by 2, 4-dinitrophenol—protoplasmic streaming is retarded. In an unstirred, unoxxygenated medium streaming slows down as an oxygen gradient is established. We

thus come to the conception of a very close relationship indeed between protoplasmic streaming and oxygen consumption.

It is even doubtful whether the vital structures of the cell can be maintained anaerobically. The protoplasmic structure of young barley leaves placed under nitrogen breaks down so completely that the cell contents ooze into the intercellular spaces within the first 48 hours. There has been no visible loss of chlorophyll and probably no appreciable proteolysis when this happens. This is a great contrast with events in air, where slow starvation precedes protoplasmic disruption. The nitrogen effect is exactly repeated if oxidation is inhibited by dilute cyanide (James & Hora, 1940). It would seem, therefore, that without oxidative respiration deterioration of the protoplasm sets in from the very start and that there is no true active maintenance under nitrogen, only a measureable rate of breakdown.

All these considerations lead one to the conclusion that plants are unable to use the energy of anaerobic respiration for many biological purposes. This remains true even though it includes anaerobic oxidations and a more than adequate decrease of free energy. Although they may be able to incur small 'oxygen debts', they need a continuous oxygen supply for normal running. The oxygen is rarely involved as a direct reactant, but, by one means or another, it brings into existence chains of reactions which provide the essential energy links coupling sugar degradation with each energy-consuming process. We may thus conceive numerous reactions competing for the available oxygen. The amount of energy available for any particular process will not be the whole of the free energy of the sugar simultaneously decomposed, but only that fraction of it which its specific linkage reaction can secure in competition. Nor is there any reason to suppose that the sum total of the linkage reactions will succeed in trapping all the free energy decrease into biologically useful channels. Rather have we good reason to believe (p. 235) that, even in the aerobic respiration of the more highly organized plants, the great bulk of it is wasted.

#### SUMMARY

Plant respiration is accompanied by a continuous decrease of free energy which appears to be a necessary condition for the continuance of life. The free energy is not utilized to any appreciable extent in doing work in the strict thermodynamic sense; moreover the release of total energy in respiration leads to its escape from the plant as heat to the amount of almost 100% during the mature phase. It falls short of 100% during the growth phase to the extent that energy is fixed by the reactions of synthesis. Apart from very trifling exceptions the heat is useless to the plant and is to be regarded as an end-product of metabolism.

During the course of its degradation the free energy of the sugar molecule produces numerous changes inside the living tissues. By far the most massive of these are chemical. The free energy relations of some biologically important reactions can be ascertained under a variety of conditions. When this cannot be done, the heat of reaction together with some semi-quantitative rules (p. 237) provide a useful though imperfect substitute and afford an indication whether a given reaction can take place or be coupled with other reactions. The minute subdivisions and intimate linkages of the metabolic reactions secure the performance of syntheses requiring free energy by means of reactions, usually oxidations, able to supply it. Other vital processes such as organization, salt

accumulation, cell division, protoplasmic rotation and many others involve the turn-over of much smaller amounts of energy which they obtain indirectly from respiration. The decrease of free energy may be far in excess of their requirements and yet remain wholly unavailable if the reaction-sequence is unsuitable. The large energy turn-over of anaerobic respiration is almost useless to the plant for this reason.

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## A COMPOSITE POLLEN DIAGRAM FROM CO. MEATH, IRELAND

By G. F. MITCHELL

*Trinity College, Dublin*

(With one pull out diagram)

### PRELIMINARY

Prof. Knud Jessen of Copenhagen visited Ireland in the summers of 1934 and 1935 to investigate the quaternary deposits, especially the peat deposits in which the country is so rich. Examination of the samples collected had not been completed by 1939, and the full results of Prof. Jessen's investigations cannot be available in the British Isles until after the war. A preliminary scheme of Irish forest development has been published and a series of zones into which Irish pollen diagrams may be divided indicated (Jessen, 1937). In recent years the study of post-glacial deposits has gone on energetically in England, and the purpose of the present paper is to make available (pending the publication of Prof. Jessen's material) a pollen diagram from the east of Ireland which appears to be typical for that part of the island.

Dr Godwin has drawn up a general scheme of forest development in England and Wales based on pollen diagrams covering the whole post-glacial period (Godwin, 1940). The diagrams have been divided into a series of zones. Unfortunately, the notation of the Irish zones does not coincide with that of the English zones, though the zones themselves (based as they are on stages of forest development) are largely in agreement. In the present paper the word 'phase' will be used as a synonym for 'zone'. In this way the confusion, which it is hoped will be removed by bringing the Irish notation into agreement with the English, will not be increased. The word 'pollen-horizon' will be used to describe a level in a pollen diagram defined by a certain relation between the tree pollens.

### THE DIAGRAM

The existence of a crannog in a drained lake basin to the east of Dunshaughlin, Co. Meath, was first noted over a century ago (Wilde, 1840). The crannog was excavated by the Harvard Archaeological Mission in 1934, 1935 and 1936, and at the same time the deposits in the basin were examined by the author, principally by a series of borings across the basin running through the crannog. The author is much indebted to Dr H. O'N. Hencken and the other members of the American Mission for assistance in the field. An account of the stratigraphy (illustrated by pollen diagrams) has already been published (Mitchell, 1940). The country in the vicinity of the basin, which lies at about 350 ft. o.d., is built up of undulating ridges of boulder clay rich in limestone. The deposits in the basin are chiefly lacustrine, but marginally there is a superficial layer of fen peat. None of the borings suggested that trees of the 'carr' type had ever invaded the margin of the fen, and the pollen diagrams seem to be free of major local distortions, though the slopes surrounding the basin were doubtless well wooded.

The pollen diagrams indicated that the rates of deposition in different parts of the basin had varied widely. While no single diagram showed a continuous record from the early post-glacial period till *ca.* A.D. 1800 when the basin was drained, each stage of forest development was illustrated by at least two diagrams, between which there was always a close agreement. It seemed therefore that a composite diagram giving the complete record could be prepared by arranging certain shorter diagrams serially.

As the rate of deposition throughout the period had not been constant, it was obvious that it would be misleading to relate the vertical height (and consequently apparent duration) of each phase in the diagram to the thickness of deposit that had accumulated at the site of the particular samples chosen to represent it. Two alternatives suggested themselves. The first was to allot to each phase the same vertical height with the consequent disadvantage that the duration of each phase would appear to be the same, which is certainly not the case. The second was to assume that certain well-marked features in Irish post-glacial development were at least approximately contemporaneous with similar features in Scandinavia, to arrange the Meath pollen-horizons corresponding to these features at vertical distances from one another proportional to the time that appears to have separated the features in Scandinavia and to construct the diagram round this framework.

The second alternative was adopted. The features selected were (i) the late-glacial solifluction deposit, assumed to be contemporaneous with the upper *Dryas* clay of Denmark (Jessen & Farrington, 1938); (ii) the increase of *Corylus* to a maximum, assumed to be contemporaneous with the beginning of the Boreal maximum of north-west Europe (Erdtman, 1928); (iii) the end of the *Corylus* maximum, assumed to be contemporaneous with the beginning of the Baltic *Littorina* transgression (Jessen, 1940); and (iv) the abrupt and widespread appearance of fresh *Sphagnum* peat, assumed to be contemporaneous with the *Grenshorizont* of the bogs of north-west Europe (Jessen, 1934). The corresponding pollen-horizons were arranged at the same distances from one another as separate the features in Jessen's scheme of post-glacial development in Denmark (Jessen, 1939). The late-glacial horizon lies at the base of the diagram (Fig. 1), the other pollen-horizons are indicated by horizontal lines, and the top of the diagram corresponds with the date A.D. 1800. The analyses falling between any two of the selected pollen-horizons were arranged at equal distances from one another. This spacing makes it appear that the spacing of the samples (from which the analyses were prepared) differed at different levels; 10 cm. was the maximum interval between any two samples.

The composite diagram was prepared from the following diagrams of the original paper; Fig. 5 (in part), Fig. 6, Fig. 8 (in part), and Fig. 9. The extent to which assumption and adjustment have altered the original data may be seen by comparing the composite with the original diagrams. Other examples of the adjustment of pollen diagrams to an arbitrary length may be seen in a paper by Rudolph (1930).

#### DISCUSSION

Irish diagrams from late-glacial deposits have already been discussed (Jessen & Farrington, 1938); as the corresponding deposits in England have yet to be elucidated, the late-glacial phases have been omitted from the diagram, which begins at the beginning of the post-glacial period proper.

The first phase is marked by the dominance of *Betula*, *Pinus* and *Salix* being the only

other trees represented; non-tree-pollen (not shown in the diagram) is abundant. The phase is referred to as Zone IV in England and Wales (Godwin, 1940) and in Ireland (Jessen, 1937). In Ireland *Pinus* tends to fall as the phase progresses, and Jessen has suggested (*in litt.*) that this may be due to over-representation of *Pinus* at the beginning of the phase due to transport of this pollen from a distance. In England, on the other hand, *Pinus* tends to rise, especially in the south and east.

The beginning of the next phase is marked by the appearance of *Corylus* pollen in considerable amounts. *Corylus* increases rapidly throughout the phase. In England the rise in *Pinus* continues, and in the south and east it displaces *Betula* from the dominant position; in the north and west the increase in *Pinus* is less marked, while in Ireland, though there is a tendency for *Pinus* to rise to a maximum, the position of *Betula* is never seriously challenged. *Salix* is reduced and the N.T.P. retreats very markedly. The phase, known in England as Zone V and in Ireland as Zone Va, is brought to a close with the appearance of *Ulmus* in quantity. At the same time there is frequently a *Pinus* maximum.

In the next phase *Ulmus* rises rapidly to importance and *Corylus* is maintained at a very high level. In Ireland values over 1000% are regularly recorded. *Quercus* appears in quantity. *Betula* undergoes a marked reduction and *Pinus* also falls back. *Salix* retreats to an insignificance from which it never again emerges. The phase is known in England as Zone VIa and forms in Ireland the lower part of Zone Vb.

The rise of *Quercus* to a maximum marks the next phase, Zone VIb in England and a later stage of Zone Vb in Ireland. *Ulmus* and *Corylus* fall back slightly from their previous values and *Betula* and *Pinus* continue at low values. At the end of the phase *Ulmus* climbs once more, but *Corylus* commences a rapid fall.

The next phase is marked by a pronounced minimum in *Corylus*, a reduction in *Ulmus* and *Quercus*, a marked maximum for *Pinus* and the appearance of *Alnus* in some quantity. The phase is recognized in England as Zone VIc but has not hitherto been recognized in Ireland where it occurs at the transition between Zone Vb and Zone VI.

*Alnus* climbs steadily to a position of importance and throughout the next phase (Zone VII in England and Zone VI in Ireland) *Alnus*, *Quercus*, and *Ulmus* maintain considerable values, while *Betula* and *Pinus* are of lesser importance. The climatic optimum falls within this phase, and in the absence of *Tilia*, *Carpinus* and *Fagus* it is difficult to trace well-defined phases in the subsequent deterioration of the Irish forests. At a time earlier than that marked by the sudden renewal of growth in the raised-bogs *Pinus* virtually disappears from Irish diagrams. The disappearance is often preceded by a small but well-defined *Pinus* maximum (not seen in the Meath diagram). It is probable that this temporary increase in *Pinus* is the equivalent of the *Pinus* maximum that marks the beginning of the English Zone VII/VIII.

Jessen has pointed out (*in litt.*) that in Ireland the subsequent reduction in *Pinus* is usually associated with a maximum for *Quercus* (sometimes accompanied by a rise in *Betula*), while *Alnus* and sometimes *Ulmus* are reduced. Such a horizon is clearly seen in the Meath diagram bringing the Irish Zone VI to an end. A similar horizon is suggested in diagrams from Shropshire (Hardy, 1939) and Wales (Godwin & Mitchell, 1938; Hyde, 1940). The horizon lies below the renewal of the growth of *Sphagnum* peat.

In the latest phase of several Irish pollen diagrams there is a pronounced rise in *Betula*, but neither the beginning of the rise nor its completion has as yet been closely linked with the behaviour of the other tree pollens. In his Irish material Jessen recognizes a Zone



VIII in which *Betula* has returned to importance. In England a similar rise in *Betula* occurs and the associated behaviour of *Carpinus* and *Fagus* enables a reasonably precise horizon to be established; here Zone VII/VIII ends and Zone VIII begins.

Comparing the post-glacial forest development in the east of Ireland, as typified by the Meath diagram, with the corresponding development in England and Wales, it is apparent that as long as the post-glacial climate was improving forest development in each area followed closely parallel courses, doubtless in response to the climatic factor. But as soon as the optimum had been passed the courses ceased to be parallel, and each became much less clearly defined. Climatic deterioration may have allowed an added importance to local factors. Godwin has made a similar observation on the relation between the forest history of England and Wales and that of the Continent (Godwin, 1934).

In the English Zone VII, immediately the optimum has been passed *Ulmus* shows a considerable reduction in quantity; in the east of Ireland no similar movement can be traced. In England there is some consistency in the later movements of *Ulmus*; in Ireland no such consistency has as yet been established. In England *Pinus* sinks to a low level before Zone VII is far advanced; in Ireland it is often maintained at considerable values till a later period, only sinking to insignificance a short time before the renewal of growth in the raised-bogs. In both areas there is a late return of *Betula* to importance. In England the return is accompanied by the persistent appearance of *Carpinus* and *Fagus*; in Ireland no such guides are available.

It is fortunate that *Carpinus* and *Fagus* never rose to positions of importance in the forests of England and Wales, for had they done so the small movements of the other trees would have been completely submerged. Detailed study of the late minor oscillations in both areas may yet enable correlation to be carried further forward. For the same reason it is fortunate that *Tilia* (except in East Anglia) only formed a minor component of the English woodlands. In England and Wales pollen of this tree does little more than mark the attainment of and subsequent decline from the climatic optimum.

#### ARCHAEOLOGICAL HORIZONS

The crannog itself and two other isolated finds make it possible to insert three archaeological horizons in the composite diagram. The crannog, which is thought from the archaeological evidence to have been occupied from the latter part of the eighth to the latter part of the tenth century A.D., belongs to a period later than the return of *Betula* to importance. A Late Bronze Age settlement in Co. Offaly dated between the fourth and first century B.C. preceded the return of *Betula* to importance (Hencken, 1942).

A bronze spearhead, typologically not earlier than Middle Bronze Age and perhaps considerably later, lies immediately above the horizon contemporaneous with the renewal of growth in the raised bogs (Fig. 12, Mitchell, 1940). No Late Bronze Age objects have yet been dated in Ireland to horizons preceding this renewal of growth, and six Late Bronze Age objects have been dated to horizons succeeding the renewal of growth.

A sherd of pottery with Neolithic 'A' affinities occurs at the horizon where *Pinus* dwindles to insignificance (Fig. 11, Mitchell, 1940). Owing to the slowness of deposition at the point where the sherd was found, its exact position in the composite diagram is a matter of difficulty: it cannot be older than the position shown. A few Neolithic, Early Bronze and Middle Bronze Age objects have been dated to horizons preceding the renewal of growth in the raised bogs. The pollen-horizons associated with finds of these

NR. DUNSHAUGHLIN, CO. MEATH



Fig. 1. A composite pollen diagram from near Dunshaughlin, Co. Meath, extending from the beginning of the post-glacial period till ca. A.D. 1800.

# REPRODUCTION IN *DRAPARNALDIOPSIS INDICA* BHARADWAJA

BY RAMA NAGINA SINGH, M.Sc.

Department of Botany, Benares Hindu University

(With 59 figures in the text)

## INTRODUCTION

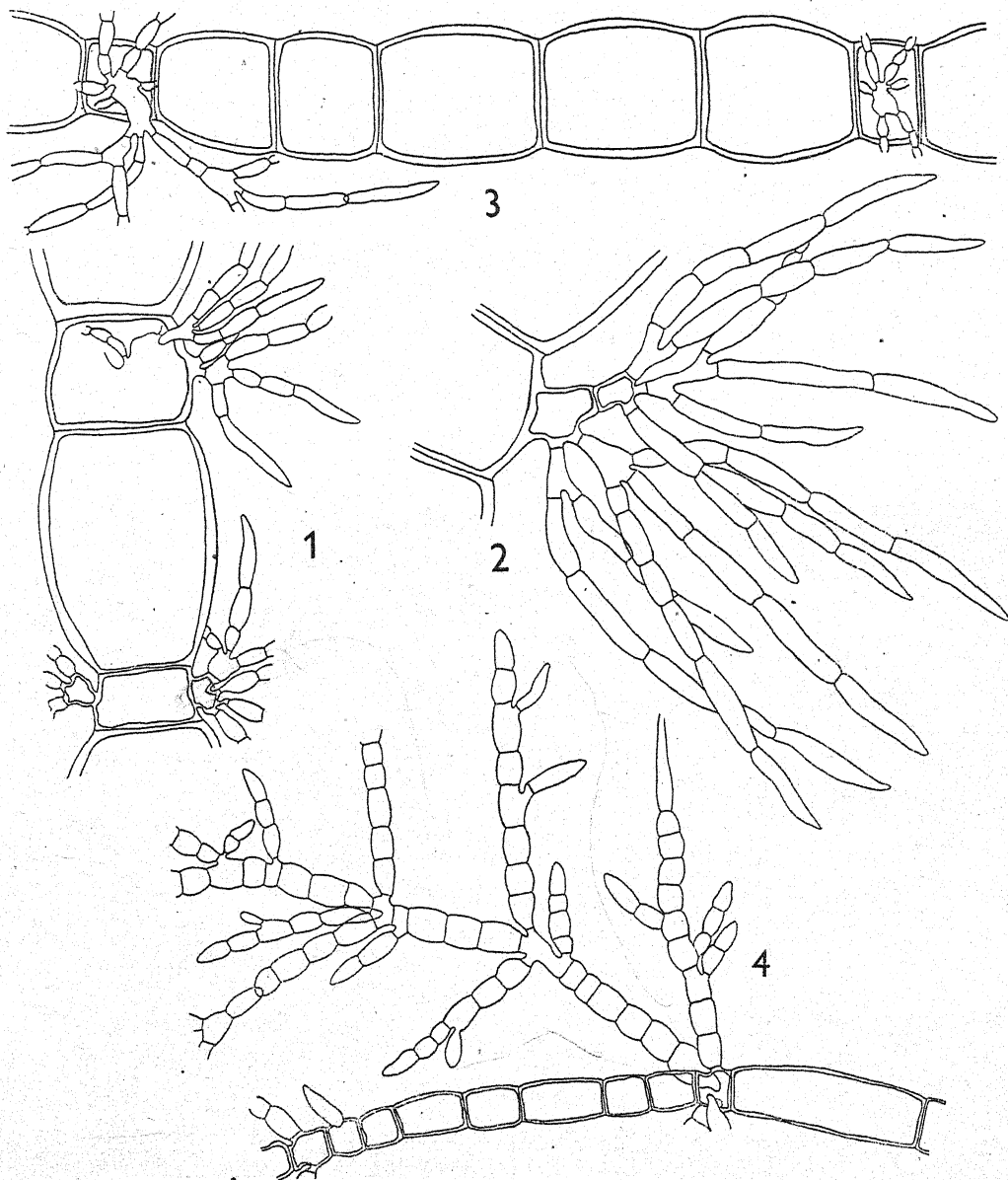
The genus *Draparnaldiopsis* was established by Smith & Klyver in 1929 on the basis of the study of a single species (*D. alpinus*) from the U.S.A. The Indian species, i.e. *D. indica*, described by Prof. Bharadwaja in 1933, was collected from a shallow pond at Benares in August 1931. As the Indian form differed from the American one in some respects, the diagnosis of the genus was amended to include the Indian species (cf. Bharadwaja, 1933, p. 9). *D. indica* Bharadwaja has by now been collected from many places in the United Provinces. It has also been found growing in some parts of Assam (cf. Parukutty, 1939) and at Lonavla, Poona District.\* Except for those mentioned no other species of the genus has been discovered.

✓ In recent years *Draparnaldiopsis* has aroused considerable interest (cf. Fritsch, 1935, p. 254) on account of certain peculiarities, viz. (1) differentiation of the filamentous plant body into main axis bearing long and short laterals of unlimited and limited growth respectively; (2) differentiation of the main axis into alternately arranged long internodal and short nodal cells; (3) long laterals repeating the structure of the main axis, and the short laterals showing no evidence of an axis and being broadly orbicular owing to the spreading nature of their subulate branches that terminate in long hairs; (4) the origin of both kinds of laterals from the median portion of the nodal cells only, the long laterals to the number of one to four and the short ones in pairs or in whorls of four; (5) production of branched rhizoids from the basal cells of the main axis (generally arising as independent structures and sometimes representing modified short laterals), as well as from any part of a lateral of limited growth and only occasionally from internodal cells, sometimes forming a dense cortical covering around the main axis and the bases of the branches (Figs. 1-4) (cf. Bharadwaja, 1933, Text-fig. 2 A-G; Pl. VIII, figs. 2-4). This genus, perhaps, exhibits the highest differentiation of the plant body among the Chaetophorales. So far only the vegetative parts of the plant have been described and the reproduction has not been studied. It is with this that the present investigation is mainly concerned.

The material collected by the writer was from a shallow pond near the village of Pahari, about four miles from the Benares Hindu University grounds, where it was growing profusely on aquatic angiosperms. In the initial stages, the habit of the plant is almost like that of a *Chaetophora*; later the gelatinous balls enlarge and become more diffuent. Finally, the alga presents a dark green beaded appearance owing to the formation of a large number of laterals from the nodal cells, thus simulating the habit of a

\* The writer has received specimens from this locality collected by Prof. V. V. Apte of Fergusson College, Poona.

*Batrachospermum*. The living alga examined under the microscope at 8.30 a.m. showed numerous swimmers outside the plant, some actively moving, while others had ceased to



Figs. 1-4. *Draparnaldiopsis indica* Bharad. 1, part of the main axis, showing the alternately arranged nodal and internodal cells and the development of the short laterals from the median portions of the former; 2, part of a nodal cell, showing the origin of short laterals from its median portion; 3, part of the main axis, showing several internodal between two nodal cells; 4, part of a long lateral of the 1st order, showing the formation of similar laterals of other orders. All  $\times 800$ .

move. In certain cells of both the short and long laterals the deep green granular contents had divided. A portion of the material was kept in pond water, while some plants were cultured in the following medium (cf. Kufferath, 1929, p. 60): soil extract, 1000 c.c.;



$\text{Ca}(\text{NO}_3)_2$ , 0.25 g.;  $\text{K}_2\text{HPO}_4$ , 0.06 g.;  $\text{MgSO}_4$ , 0.06 g.;  $\text{KNO}_3$ , 0.06 g.; trace of ferrous sulphate. The soil extract was prepared by boiling 1 kg. of soil with 1 kg. of water for 2 hr. under a reflux condenser. The extract was allowed to settle for at least a fortnight before it was decanted off. The cultures were made in a solid medium, obtained by adding 3% agar to the above solution in Petri dishes. Plants were inserted in the agar while it was setting. After the agar had set, it was well covered with more of the culture medium. Material thus grown did not, in general, form zoospores. Pieces of old plants were therefore removed from the agar on which they were growing and inoculated into fresh agar, so that they were not deeply embedded. They were then covered as usual with more of the culture medium. This procedure proved quite successful, for on the morning after inoculation a large number of laterals of different orders formed zoospores. Material grown in pond water also formed zoospores after about 24 hr., but these plants did not survive long. The observations given below were made on plants grown in cultures and also on living material freshly collected from the pond. The ecological factors were investigated in the field, excepting the redox potential and potentiometric pH value determinations.

#### FACTORS CONTROLLING SWARMER-FORMATION

During the rainy season (July-Sept.), when the present investigation was carried out, swarmer formation takes place early in the morning. It starts at about 6 a.m. and continues up to 9.30 a.m.; but if the cultures are kept in the dark, it can be delayed for 2 or 3 hr. and will occur within a few minutes of bringing them into the light at any time between 6 and 11 a.m. On the other hand, if cultures are kept in the dark for a longer time, swarmer formation ensues while they are still in the dark, and generally between 10 a.m. and 12 noon. Under these circumstances, however, many of the zoospores do not escape from the cells, but develop as thick-walled hypnospores.

The pH of the medium is an important limiting variable controlling swarmer formation. An alkaline medium is quite favourable for the initiation of the process. From 6 to 8.30 a.m., the pH of the pond water in nature, as determined with the help of a B.D.H. universal indicator and checked by the potentiometric observations in the laboratory, is between 7.2 and 7.5, and this appears to favour the production of macrozoospores and gametes. After 8.30 a.m., when the pH increases from 7.5 to 8.5, the formation of these two types of swarmers is arrested and microzoospores are produced.

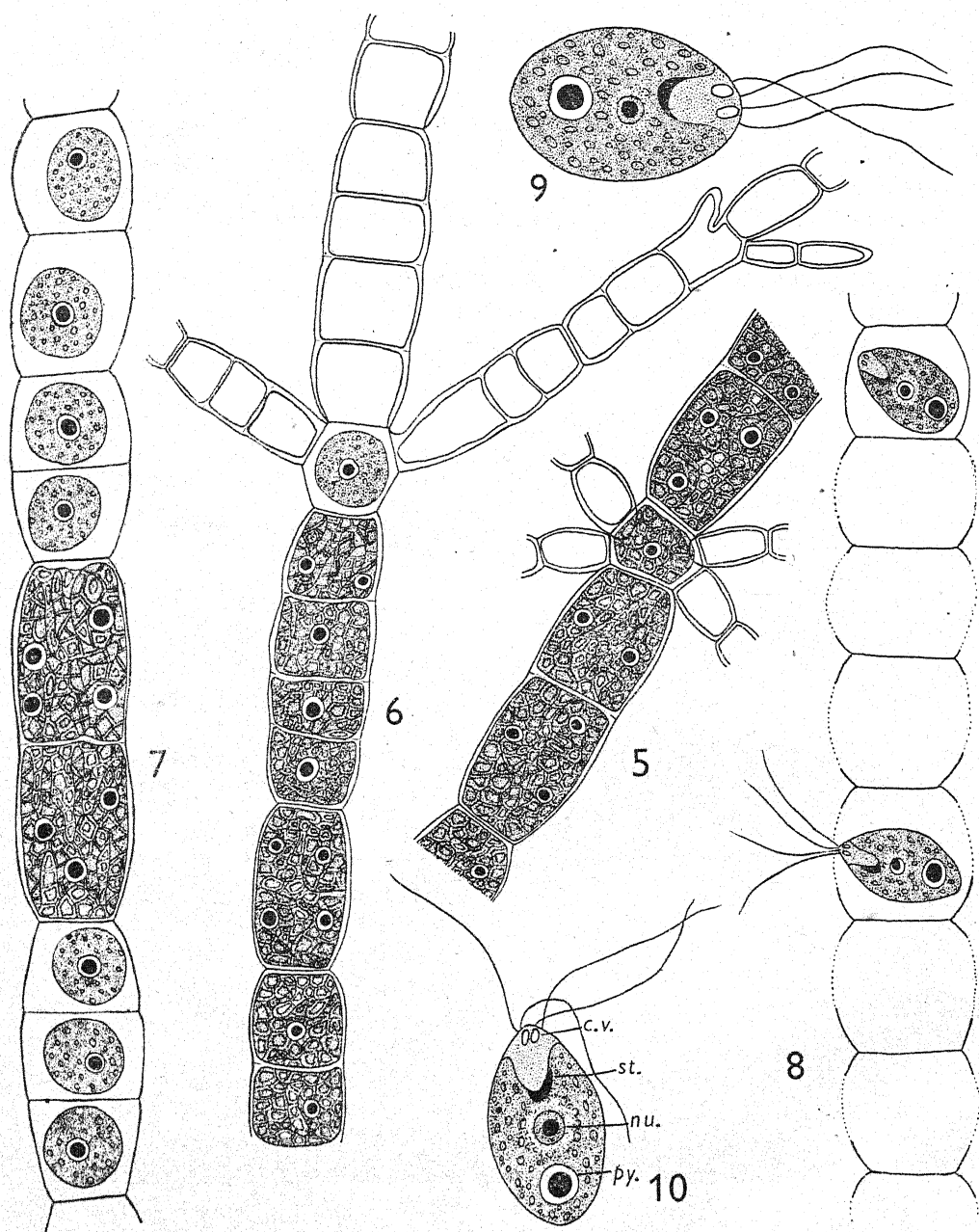
At the time of formation of macrozoospores and gametes the redox potential of the pond water varied between 310 and 340 mV. ( $E_7$  values). The modified ammonium thiocyanate test resulted in the reductivity being two and the diphenylamine test for nitrates was negative in response. A reducing condition of the medium therefore obtained during the formation of these two types of swarmers. On the other hand, microzoospores were formed when the oxidative intensity was high. The  $E_7$  values, at this time, varied between 380 and 410 mV. The ammonium thiocyanate solution gave a slight red colour which persisted even after the addition of hydrogen peroxide, possessing almost the same intensity. The diphenylamine test gave a slight blue coloration. The explanation for this no doubt lies in the enhanced photosynthetic activity of the alga so that more and more oxygen is being liberated, which promotes oxidative processes.

## PRODUCTION OF SWARMERS AND THEIR LIBERATION

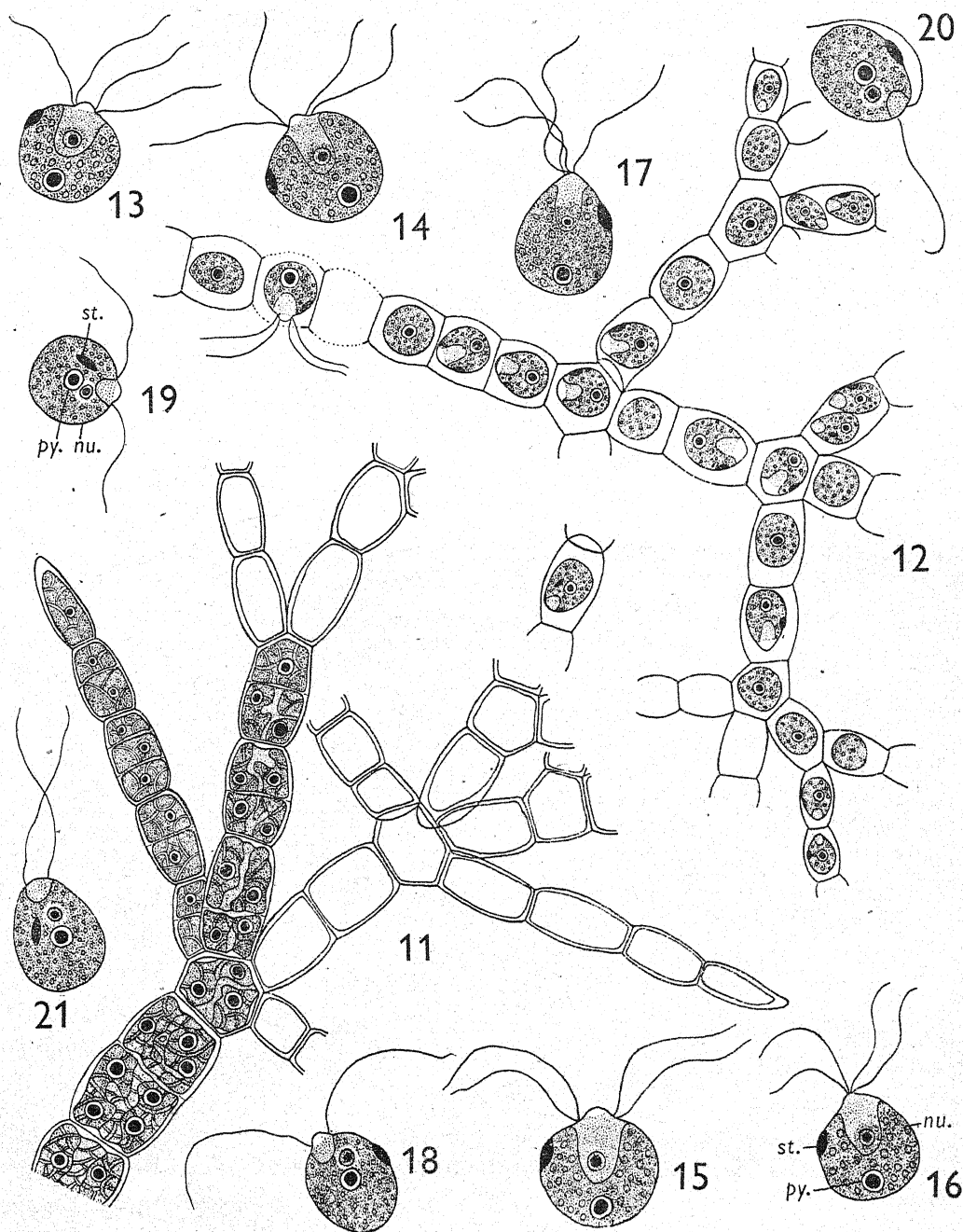
Swarmers formation is confined to the laterals, as in *Draparnaldia plumosa* Ag. (Johnson, 1893, p. 295, Pl. XXXII, fig. 2). During the 24 hr. preceding the actual commencement of the process, most of the cells of both the long and short laterals, excepting the nodal cells of the former and the terminal hair cells of the latter, undergo a division, which is accompanied by the formation of a thin transverse wall (Figs. 5, 11). Such walls do not constrict the filament to the same extent as the original cross-walls. A second transverse division (Figs. 6, 11 and 23), and sometimes a third one, may also occur. Thus, in the filaments which are about to form swarmers, the cells usually appear in distinct pairs or in groups of four or more (Figs. 6, 11 and 23). Each such group shows a fairly thick septum at either end where the filament is considerably constricted, while the individual cells of the group are separated by very thin and only slightly constricted walls (Figs. 6, 11 and 23). The typical appearance of the plant is thus greatly altered, while the alternate arrangement of nodal and internodal cells in the long laterals, and occasionally in the main axis also, is lost (Figs. 3-7, 11 and 23). The several cells thus formed between two nodal cells no doubt represent the products of repeated division of the original single internodal cell. The nodal cells vary in shape and size. They are both short and long, discoidal and barrel-shaped, rhomboidal or polygonal (Figs. 4-6, 11, 12 and 22-24). The branches do not always arise from the middle of these cells but may originate from any part (Figs. 4-6, 11, 12 and 22-24). They sometimes arise in groups of more than four (Figs. 4, 11), as is especially the case at the apices of the long laterals (Fig. 23).

The first sign of the imminence of swarmer formation is a gradual elongation and turgidity of the contents of the reproductive cells which enlarge with great rapidity (Figs. 5, 6, 11 and 23). While the newly formed transverse walls increase in width, the older cross-walls stretch only slightly, no doubt because of their greater thickness (Figs. 5-7). Consequently, each group of cells becomes markedly barrel-shaped, with the widening walls separating the individual cells of the group becoming so thin as to be almost indistinct. Slight constrictions, however, indicate their position. Simultaneously the protoplast begins to round off and recede from the cell wall so that the filament ultimately contains a number of rounded protoplasts equal in number to the newly formed cells (Figs. 7, 12 and 23). In the next stage the rounded protoplasts acquire the characteristic shape of the swarmers and the flagella develop. Liberation of the swarmers takes place by the complete gelatinization of the longitudinal walls (Figs. 8, 12). The lateral branches are often almost completely used up in this process, as has been recorded in *Draparnaldia plumosa* Ag. (Johnson, 1893, p. 295, Pl. XXXII, fig. 2) and in *D. glomerata* Ag. (Fritsch, 1935, p. 257), and after the swarmers have been liberated, only the remnants of the transverse walls, both old and new, are to be seen (Figs. 8, 24).

Three types of swarmers, similar to those found in *Ulothrix* (especially *U. zonata* Kütz.) and in *Fritschella tuberosa* Iyeng. (Singh, 1941), have been recognized, viz. quadriflagellate macrozoospores, quadri- or biflagellate microzoospores, and biflagellate gametes. The quadriflagellate macro- and microzoospores are formed singly in each daughter cell, whereas the biflagellate microzoospores and the gametes may be formed singly or in pairs, threes, fours or more, as in *Draparnaldia glomerata* Ag. (Fritsch, 1935, p. 257; Ferguson, 1932). The formation of the various types of swarmers is confined to branches of specific orders. The macrozoospores are invariably formed by the long



Figs. 5-10. *Draparnaldiopsis indica* Bharad. 5, part of long lateral of first order, showing first division during macrozoospore formation; 6, the same showing second division and arrangement of cells in groups; 7, showing rounded protoplasts, one in each daughter cell; 8, showing gelatinization of longitudinal walls and liberation of spores; 9, 10, macrozoospores. *c.v.* contractile vacuoles; *nu.* nucleus; *py.* pyrenoid; *st.* stigma. 5-8,  $\times 1600$ ; 9, 10,  $\times 3400$ .



Figs. 11-21. *Draparnaldiopsis indica* Bharad. 11, part of a long lateral, showing division of cells; 12, the same showing formation of microzoospores; 13-15, spherical, and 16, 17, pear-shaped, quadriflagellate microzoospores; 18, subspherical, 19, spherical, 20, ovoid, and 21, obvoid biflagellate microzoospores. nu. nucleus; py. pyrenoid; st. stigma. 11 and 12,  $\times 1600$ ; 13-21,  $\times 3400$ .

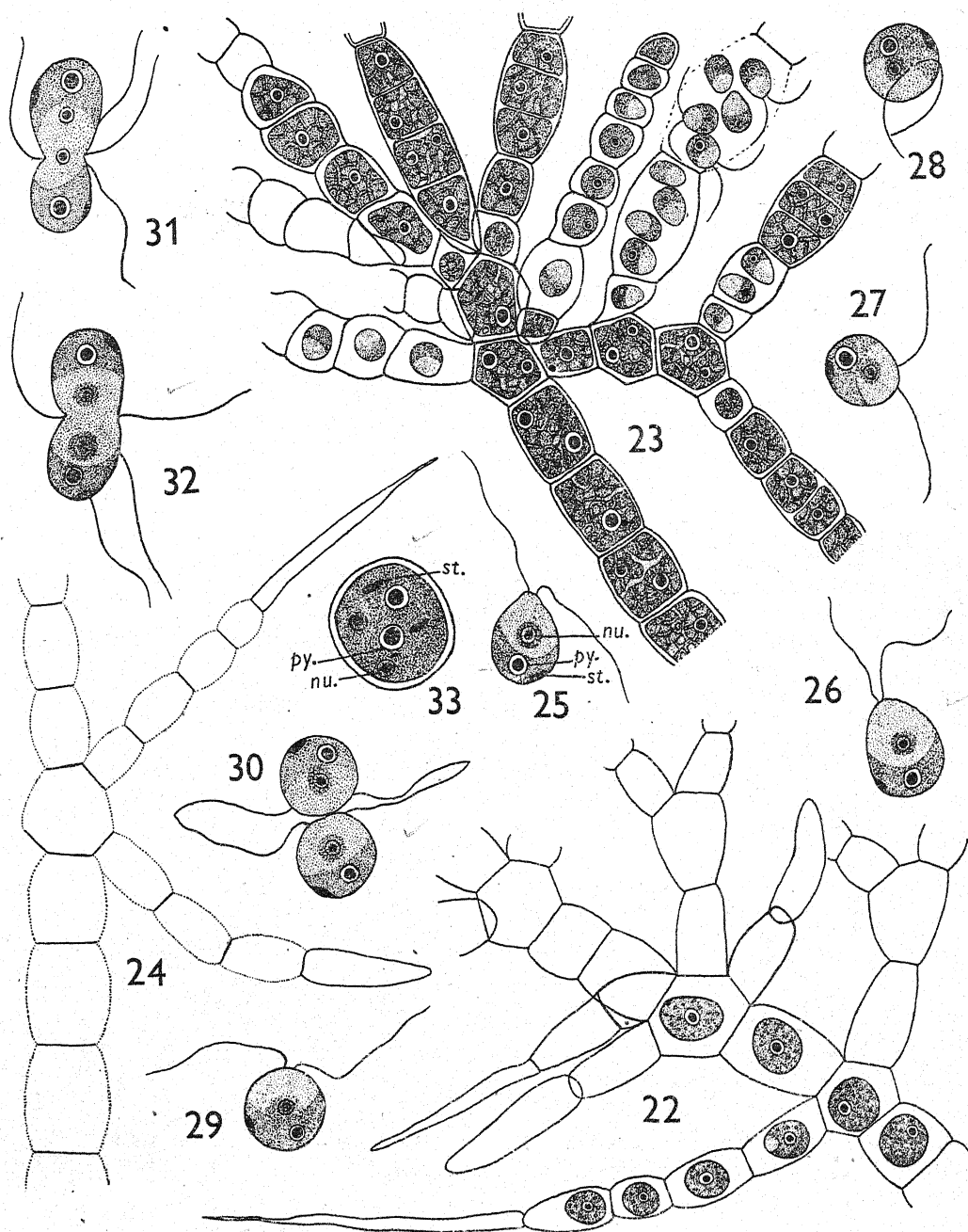


laterals of the first order (Figs. 6-8), while the production of microzoospores is restricted to similar laterals of other orders, as well as to short laterals of all orders. Again, the quadriflagellate microzoospores are formed by the long laterals only and the biflagellate ones by short laterals (Fig. 12). The gametes are formed by the short laterals of the last two orders (Fig. 23). There is therefore a marked division of labour so far as the production of the different types of swimmers is concerned. Moreover, macro- and microzoospores are formed on the same plant, while gametes are formed on a different one, which is otherwise quite similar to the former.

The macrozoospores are ellipsoid or ovoid (Figs. 9, 10, 34 and 35), and measure  $12-17.3\mu$  in length and  $6.7-10.6\mu$  in breadth. The four equal flagella arise anteriorly, but somewhat subterminally. The red eye-spot is flattened and more or less median, usually somewhat nearer to the anterior end. There is a single basin-shaped chloroplast, possessing a large pyrenoid near the posterior end. The single nucleus is situated in the centre. There are two distinct contractile vacuoles just below the point of attachment of the flagella. The macrozoospore of this alga is thus very similar in shape and structure to that of *D. plumosa* Ag. and *D. glomerata* Ag. (Johnson, 1893, p. 295, Pl. XXXII, fig. 1; Fritsch, 1935, p. 256, Fig. 76 C). The quadriflagellate microzoospores are more or less spherical, flattened and somewhat bulged in the middle and possess a clear anterior protoplasmic papilla, around which the four equal flagella arise (Figs. 13-15). Some are, however, pear-shaped with the anterior end drawn out to a point, from which the flagella arise (Figs. 16, 17). They measure  $7-8.8\mu$  in diameter. There is a single deeply notched chloroplast, possessing a pyrenoid at the posterior end. The projecting stigma is nearer the anterior end, and the nucleus is located almost in the centre. There is considerable variation as regards the shape and dimensions of the biflagellate microzoospores, so that at times it becomes very difficult to distinguish some of them from the gametes. They are usually spherical or subspherical, but sometimes ovoid or obovoid (Figs. 18-21). The projecting stigma is situated near the middle. The single chloroplast is slightly notched and possesses a pyrenoid at its centre. The nucleus is slightly above the pyrenoid. The two equal flagella arise anteriorly and sometimes on either side of a protoplasmic papilla. These microzoospores are  $6.5-8\mu$  long and  $4-5.3\mu$  broad. The biflagellate gametes are spherical or in some cases ovoid, with the flagella arising anteriorly. The slightly notched chloroplast is located at the posterior end of the swimmer, occupying usually about one-third of the whole contents (Figs. 25-29). There is a single pyrenoid. The linear eye-spot lies laterally at the posterior end and the nucleus is placed more or less centrally in the hyaline anterior portion. The gametes measure  $5.3-6.7\mu$  in length and  $4-5.3\mu$  in breadth. The dimensions of all the swimmers, however, vary about their own mean.

#### GERMINATION OF THE ZOOSPORES

The macro- and microzoospores, after moving for a period which does not exceed 8 hr. in the case of the former and up to 24 hr. in that of the latter, become negatively phototactic and settle down on the side of the culture dish away from the light, where they become attached by their anterior ends. The flagella are lost, and the cell rounds off and becomes enveloped by a delicate wall (Fig. 36). The swimmer then begins to form, at the attached end, a hyaline protuberance which gradually elongates in a lateral direction (Figs. 37-39, 43-47, 49-50 and 52-53) and becomes cut off by a transverse wall near its base to form a rhizoidal cell. This grows into a filamentous rhizoid with scanty contents



Figs. 22-33. *Draparnaldiopsis indica* Bharad. 22, part of long lateral of second order, showing formation of microzoospores; 23, apical part of a long lateral, showing conversion of the cells of short laterals into gametes; 24, part of long lateral after the liberation of swimmers; 25-29, gametes; 30-32, conjugating gametes; and 33, zygote. nu. nucleus; py. pyrenoid; st. stigma. 22-24,  $\times 1600$ ; 25-33,  $\times 3400$ .

that ultimately disappear completely (Figs. 39-41, 48 and 51). The upper cell repeatedly divides transversely to give rise to a short green filament of 3-4 or more cells, each containing a band-shaped chloroplast with two or three pyrenoids (Figs. 40, 48 and 51). The filaments produced by the biflagellate microzoospores are comparatively narrower and shorter than those produced by other swimmers (Fig. 51). The new filament, in course of time, grows in length by repeated cell division to form a long thread of over a hundred similar cells, while the rhizoid extends by repeated forking and branching (Figs. 41, 42). After a considerable time the filament becomes differentiated into nodal and internodal cells, and laterals both of limited and unlimited growth begin to arise.

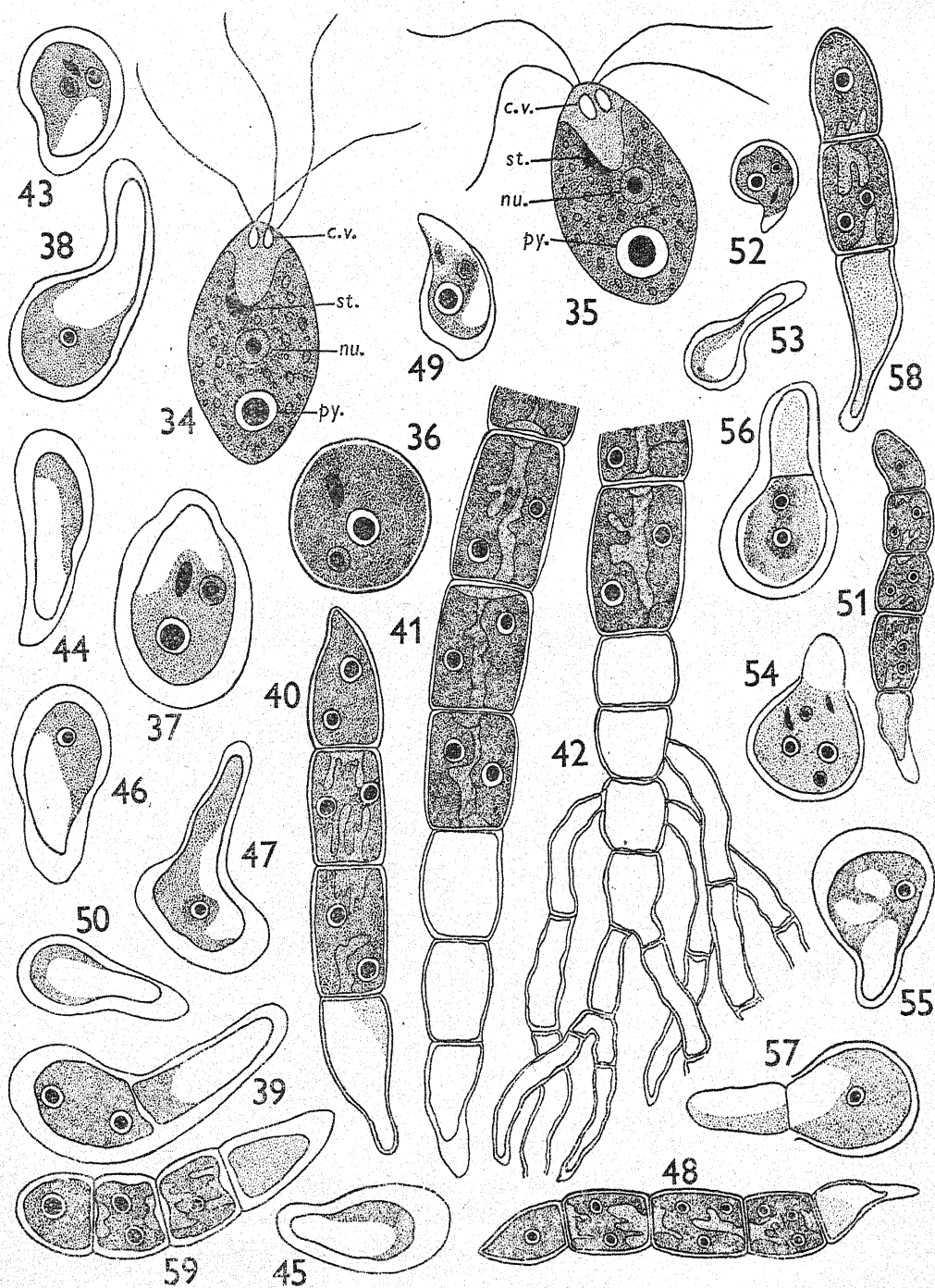
#### CONJUGATION OF THE GAMETES AND THE DEVELOPMENT OF THE ZYGOTE

Gametes belonging to the same plant do not, in general, fuse with one another. When a drop of water containing gametes from one plant is mixed with a drop containing gametes from another, the gametes begin to pair. The pairing gametes face each other by their anterior ends, become entangled by their flagella, and finally fuse (Figs. 30-32). Immediately after conjugation the product becomes negatively phototactic and moves towards the less illuminated side of the drop. The conjugating gametes are usually very similar to one another (Figs. 30, 32), but occasionally one is slightly bigger than the other (Fig. 31). The process of fusion proceeds gradually and takes from a few minutes to sometimes nearly half an hour. The zygotes usually remain motile for a short time, after which they attach themselves to the sides of the dish, lose their flagella, and gradually round off. At this stage the contents exhibit clearly the two nuclei, the two pyrenoids and the two eye-spots derived from the gametes (Fig. 33). The pyrenoids and nuclei fuse and at the same time the eye-spots disappear. The zygote ultimately surrounds itself with a delicate wall, usually within 4-6 hr.

The zygote does not undergo any resting period and may commence to germinate even after 24 hr. It loses its spherical shape, and becomes somewhat ovoid. After another 24 hr. it produces a hyaline protuberance at one end (Figs. 54, 55), while on the third day it consists of two cells (Figs. 56, 57), the one, presumably basal, having meagre contents and the other possessing a well-developed chloroplast with one or two large pyrenoids. After 5 or 6 days filament of 4-6 cells is formed (Figs. 58, 59). Further development takes place rather rapidly, and in course of time a long thread consisting of about a hundred similar cells is produced, while the basal rhizoidal cell becomes markedly slender and branches repeatedly to give rise to a tuft of rhizoids. The further development is similar to that met with during the germination of the macrozoospores.

#### DISCUSSION

It is evident that *Draparnaldiopsis indica* Bharadwaja possesses well-defined asexual and sexual methods of reproduction, effected by means of motile swimmers. In the production of three types of swimmers, viz. quadriflagellate macrozoospores, quadri- or biflagellate microzoospores, and biflagellate gametes, the alga resembles *Ulothrix* (especially *U. zonata* Kütz.) and *Frittschiella tuberosa* Iyeng. (Singh, 1941). In the possession of large asexual quadriflagellate macrozoospores it resembles the members of the Chaetophoreae in which reproduction has been studied, excepting the genera *Iwanoffia* and *Pilinia*, where the macrozoospores are biflagellate (Fritsch, 1935, p. 255). It agrees with *Stigeoclonium* and *Draparnaldia* in the production of quadriflagellate microzoospores and with



Figs. 34-59. *Draparnaldiopsis indica* Bharad. 34, 35, macrozoospores; 36, resting macrozoospore; 37-39, stages in the germination of macrozoospore; 40, a 5-day-old germling, showing the basal rhizoidal cell; 41, a part of a germling with filamentous rhizoid; 42, the same as in (41), showing branching of rhizoid; 43-47, stages in the germination of quadriflagellate microzoospores; 48, a 6-day-old germling formed on germination of a quadriflagellate microzoospore; 49-51, stages in the germination of biflagellate microzoospores; 52, 53, the same of another type of biflagellate microzoospores; 54-59, stages in the germination of zygote. *c.v.* contractile vacuole; *nu.* nucleus; *py.* pyrenoid; *st.* stigma. All  $\times 3400$ .



other members of the above subfamily in its biflagellate microzoospores. It differs, however, from these genera, apart from the species *Stigeoclonium longipilum* and *S. fasciculare* (Fritsch, 1935, p. 255), in the possession of a third type of swarmer, which is invariably biflagellate and differs from the biflagellate microzoospores, and which in its morphological features recalls the gametes of *Ulothrix zonata* Kütz. and *Frittschiella tuberosa* Iyeng. (Singh, 1941). *Draparnaldiopsis indica* is quite distinct from the forms mentioned above in the fact that its microzoospores are very variable and exhibit a gradation between macrozoospores and gametes, inasmuch as some of the biflagellate microzoospores are very similar to the gametes, both in shape and dimensions, and in some of the internal morphological features. This supports the view put forward by Chohnoky (1932), that in *Ulothrix variabilis* Kütz. and *U. zonata* Kütz. the gametes are formed from potential zoospores, and the same seems true in *U. rorida* Thur. (Lind, 1932).

The opinion which is sometimes expressed that the species which are vegetatively less specialized possess the same three types of swarmers as in the genus *Ulothrix* (cf. Fritsch, 1935, p. 255), appears to be untenable in the light of the present investigation. In *Draparnaldiopsis indica*, which perhaps represents the most highly evolved plant among the Chaetophorales, we find the same primitive type of reproduction as in *Ulothrix*, and this is also so in the terrestrial alga, *Frittschiella tuberosa* Iyeng. This behaviour is in conformity with the well-known doctrine that conservatism in reproduction is the rule among plants. The very close agreement in methods of reproduction and in the simple filamentous structure of the juvenile plants with band-shaped chloroplast in their cells, between *Draparnaldiopsis indica* and *Ulothrix*, strongly supports the probable common origin of the Ulotrichales and Chaetophorales (Fritsch, 1929, p. 111; Singh, 1941).

#### SUMMARY

The reproduction of *Draparnaldiopsis indica* Bharadwaja has been studied. Three types of swarmers, viz. quadriflagellate macrozoospores, quadri- or biflagellate microzoospores and biflagellate gametes have been recognized. The macro- and microzoospores are invariably asexual in nature, whereas the more or less similar gametes from different plants fuse in pairs to form zygotes, which germinate directly to give rise to new plants. The reproduction of this plant is, therefore, similar to that of *Ulothrix zonata* Kütz. and *Frittschiella tuberosa* Iyeng.

Some of the conditions obtaining during swarmer formation have been studied. An alkaline medium is, in general, most favourable for the process. The pH value of the water appears to be one of the limiting variables in the formation of the different types of swarmers. Macrozoospores and gametes are formed at a lower pH value than microzoospores. It is also found that while a reducing medium favours the production of the two former types of swarmers, an oxidizing medium favours the latter.

In conclusion, I have much pleasure in expressing my great indebtedness to Prof. Y. Bharadwaja for his kind guidance and criticism throughout the course of this investigation.

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# CONTRIBUTIONS TO OUR KNOWLEDGE OF BRITISH CHRYSOPHYCEAE\*

By J. W. G. LUND, M.Sc., Ph.D.

(With 11 figures in the text)

In the course of an investigation of the algal flora of the bottom sediments in a number of ponds in Richmond Park, Surrey, a number of new and interesting Chrysophyceae were studied, and it is with these that the present communication deals. The *Apistonema*, however, came from a different locality. Ecological relations of the forms here described are dealt with elsewhere (Lund, 1942).

The preservation and fixation of many Flagellates is a matter of considerable difficulty and most cannot be studied satisfactorily in formalin material, while many Chrysophyceae disintegrate more or less completely in this preservative. I have found osmic acid satisfactory as a fixative, but it is essential to remove the acid after fixation, since it almost invariably causes more or less marked blackening after a time. Such blackening sets in very rapidly in algae storing oil (Chrysophyceae) or having a protoplast rich in oil (Euglenineae). It can be obviated by immediate treatment with 4% formalin followed by washing in water on a Whatman no. 50 filter paper for not less than 6 hr. The method may be summarized as follows:

- (1) Fix in 1.0 or 0.5% osmic acid (acid and material in the ratio of 1:1 or 0.5:1).
- (2) Remove within 2 min. to a filter paper and add 4% formalin until the filter is nearly full.
- (3) Wash with water for not less than 6 hr.
- (4) Preserve in 4% formalin.

Staining was not necessary to study the salient features of the cells.

## CHROMULINA

Owing to the high degree of metaboly common to many species, and since the motile stages afford relatively few useful specific characteristics, the taxonomy of this genus is fraught with much difficulty. The most valuable characters are those provided by the cysts, but in many species these are either unknown or rarely formed and when present persist for a short period only. For a number of species, however, it has been possible to study all essential stages.

### 1. *Chromulina aerophila* n.sp. (Figs. 1, 2)

In nature this was only found in a disused brick drain connecting the two Pen Ponds, but it frequently appeared in cultures of sediments from several of the ponds in distilled water or a 0.05% Benecke solution; this implies that cysts are present in most of the sediments. The alga occurs in two somewhat different forms. That found in nature and in distilled water cultures may be described as forma A, while that occurring in cultures with a Benecke solution is described as forma B.

\* From the Department of Botany, Queen Mary College, University of London. The substance of this paper formed part of a thesis accepted by the University of London for the degree of Ph.D.

The motile individual (5–9 by 4–5 $\mu$ ) of the former is usually ellipsoidal to oval in shape (Fig. 1 A–E, H) but is somewhat metabolic; the anterior end, though generally rounded, may be flattened (Fig. 1 E) or even indented (Fig. 1 F). Cells moving sluggishly or about to cease movement are more or less spherical (Fig. 1 H). The flagellum is approximately as long as the cell, while two anterior contractile vacuoles, pulsating alternately, are present, but no stigma. The single rather variable parietal chromatophore lies along one side of the cell. It generally forms a short and wide band extending about half-way round the cell (Fig. 1 A, B, E, F), but the band may be of some length and exhibit diverse orientation with reference to the long axis of the cell (Fig. 1 A, B, F). A pyrenoid is frequently visible, especially in material killed in osmic acid, apposed to the inner surface of the chromatophore. The food reserves are leucosin and oil, and nutrition appears to be holophytic (see also below).

When there is a stable surface film, the dominant vegetative phase is non-motile, with the cells forming a coating of dust-like particles on the surface of the water. This coating appears greyish or golden, according to the angle from which it is viewed. In its production the cells swim to the surface and press themselves against the surface film, after which there begins a slow spiral movement resulting in the cells pushing through the film. The part first to project through the latter appears, when viewed from the surface under the microscope, like a small bubble occupying the middle of the cell. As the individual continues to penetrate the film, more and more of it becomes obscured until only a superficial bubble-like structure is visible. The layer of cells then appears, when seen from above, as a mass of bubbles on the surface of the water. With dark-ground illumination this emits a golden light.

The bubbles may be minute and round, but often appear relatively large and oval to vermiform or altogether irregular in shape. Microscopic observation shows that these large bubbles consist of a number of cells enclosed in a common envelope. Careful focusing of parts of the surface film, bearing bacteria or other foreign particles, proves by comparison that the *Chromulina* cells are actually above the water surface. This is also evident when material placed on a coverslip, inverted over a glass ring, is viewed from below. A side-view of the bubbles is difficult to obtain because of the refraction at the air/water surface, but a thin glass rod, gently dipped into the water film and fixed to a micro-dissection apparatus, admits of examination of the cells from the side and shows that they are raised above the water surface by means of a short stalk (Fig. 1 G, I–K, s). This stalk is simply the modified posterior end of the cell as indicated by the position of the contractile vacuoles; it is often ill-defined and only clearly marked in cells lacking a flagellum.

Since the cells swim freely as soon as submerged, they must be capable of being wetted. On the other hand, if the stalks could be wetted, it would be impossible for these small bodies to withstand the surface tension of the water film still enveloping the cell as a result of capillarity; its surface tension would again depress the cell beneath the surface. It is, therefore, clear that some part of the cell must be unwettable. Photographs,\* show that this is true of at least a portion of the stalk above the surface film, so that water is not in connexion with the cell above the stalk. The nature of the substance responsible for the unwettable character of the stalk has not been ascertained. Tests for fat and cellulose gave negative results.

\* Fig. 31 in the author's Ph.D. thesis referred to in the footnote to p. 274.



By shaking the dish, by blowing, by dropping water on to the surface, by covering the surface with a coverslip or otherwise disturbing it, the cells are immediately submerged. Such treatment results in bringing the exposed part of the cell into contact with the water by causing the stalks to lose their vertical position. This also proves that the cells can be wetted, but not the stalk. After submergence the cells, forthwith or shortly afterwards, resume motility, and investigation of fixed material from an undisturbed film shows that the majority of the cells retain their flagella during the non-motile stage. In the majority of such fixed cells the stalk is ill-defined and merely composed of the prolonged posterior end.

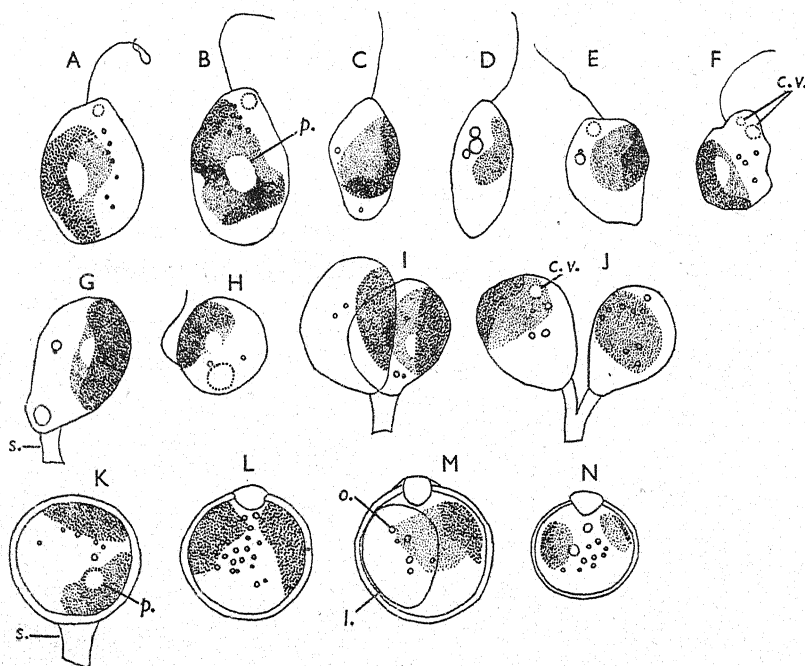


Fig. 1. *Chromulina aerophila* n.sp., forma A. A-F, H, motile cells; G, I, J, resting stages on surface film; K-N, cysts. c.v. contractile vacuole; l. leucosin; o. oil; p. pyrenoid; s. stalk. (A, B, I,  $\times 2450$ ; C-H, J-N,  $\times 2250$ .)

The envelope surrounding the cells (but not the stalks) in the non-motile stage is lost as soon as they are submerged (cf. p. 279), nor is it recognizable in the killed and stained cells; its rapid disappearance is also implied by the customary immediate resumption of motility. If a quantity of water bearing this characteristic stage of the alga is allowed to dry, the 'bubbles' representing the algal cells only dry up when the water itself has evaporated, as can be seen if the drying film on a thin glass rod attached to a microdissection apparatus is watched. When evaporation is complete the envelopes disappear, the algal cells disintegrate and the free water dries up, all at the same moment. If the envelope around the alga consisted of a substance other than water, it would almost certainly dry at a different rate than the free water and should be capable of being rendered visible after immersion in water or by staining. In bubbles containing several individuals some of them are occasionally seen swimming freely inside; the latter have become free from their stalks, the whole bubble being held above the surface by the

stalks of the remaining individuals. All the cells of such a group are never motile. It is, therefore, concluded that the envelope surrounding the alga is merely water (cf. also p. 279).

Since the water surrounding the exposed cell will gradually evaporate, it must be replenished from the cell; but the latter cannot possess enough reserves of water continuously to replace that lost by evaporation and the loss must be made good via the stalk. It remains doubtful whether the latter is actually hollow, but its surface differs from the central region, which is perhaps occupied by mucilage. The conduction of water into the exposed cell via the stalk has been demonstrated by the passage of dilute intra-vitam dyes (methylene blue or neutral red), added to the water, into the cell. A miniature transpiration system is thus established from the water through the stalk into the exposed cell, where it exudes to form the envelope and finally evaporates into the air. The process is probably relatively slow, since the air close to the water surface will be humid, and the non-motile stage can occur only in quiet weather.

The iridescence of the cells, when viewed from a certain angle, is due to the same cause as in *C. Rosanoffii* (Molisch, 1901), *C. smaragdina* (Gicklhorn, 1922), *Schistostega osmundacea* and certain other shade plants. When the coating of *Chromulina* cells is observed from the same direction as the source of light, it shows a golden iridescence; when viewed from the opposite direction, it appears grey. Orientation to a change in the direction of the light is rapid. If the dish with the alga is turned through  $180^\circ$ , the golden iridescence appears on the side facing the source of light within 2 min. Microscopic observation shows that the chromatophores of all the cells are located on the side away from the source of light. The cell thus acts as a biconvex lens concentrating the light on the chromatophore from which it is reflected.

Gicklhorn (1922) states that in *C. smaragdina* the change is due to the aggregation of the chromatophores on the side away from the light, but gives no proof that this is a result of movement of the chromatophores. It is extremely difficult to establish whether there is a movement of the chromatophores or of the whole protoplast. Although the change is rapid, the actual distance moved in a reorientation through  $180^\circ$ , even in a cell  $10\mu$  in diameter, is only about  $15.7\mu$ . I have, however, gained the impression that the protoplast moves as a whole, though the means by which this is accomplished is difficult to understand, nor has it been possible to observe any free movement when single cells constitute a bubble. When submerged by superposing a cover-glass, the cells swim actively towards the light, but if a free surface is available they at once accumulate above it, though the part nearest the source of light generally bears the greatest number of cells.

The capacity of the cell to take up a definite orientation towards the light is clearly of advantage in the natural habitat, which is comparable to the rabbit burrows and other shaded positions commonly occupied by *Schistostega*. When viewed from the opening, the surface of the water and the adjacent sides of the drain presented a beautiful golden sheen. The absence of the alga from other habitats in the Pen Ponds may be attributed to its shade-loving habit and the necessity of perfectly quiet conditions for the maintenance of the non-motile stage.

Division is restricted to this stage, two cells often possessing a common (Fig. 1 I) or branched stalk (Fig. 1 J). The cysts arise from the non-motile stage and retain the stalk which is not included in the cyst (Fig. 1 K). They are spherical or subspherical ( $6.8-8.5\mu$ )

(Fig. 1 K-N) with a smooth silicified wall and a plug which is almost flush with the surface and difficult to distinguish (Fig. 1 L-N). They usually contain two chromatophores (Fig. 1 K, L, N), although only one may be present (Fig. 1 M), as well as large quantities of oil and often a big leucosin globule.

The other form (forma B) occurred only in cultures of certain marginal sediments, being often so abundant in those treated with a 0.05% Benecke solution that the non-motile stage formed a thick brown, often wrinkled, pellicle which could be easily lifted with a pair of forceps. Forma B was present only when the water was highly acid, usually with a pH of 3.6-5.0 but occasionally with one between 5.0 and 6.0. Forma A, on the contrary, never occurs when the pH is below 5.0 and is usually found at a pH of 6.5-7.5.

The motile cells (8.5-12.0 by 3.5-7.0  $\mu$ ) resemble those of forma A, but the single chromatophore is generally more definitely band-shaped (Fig. 2 A, B, E). The oval to

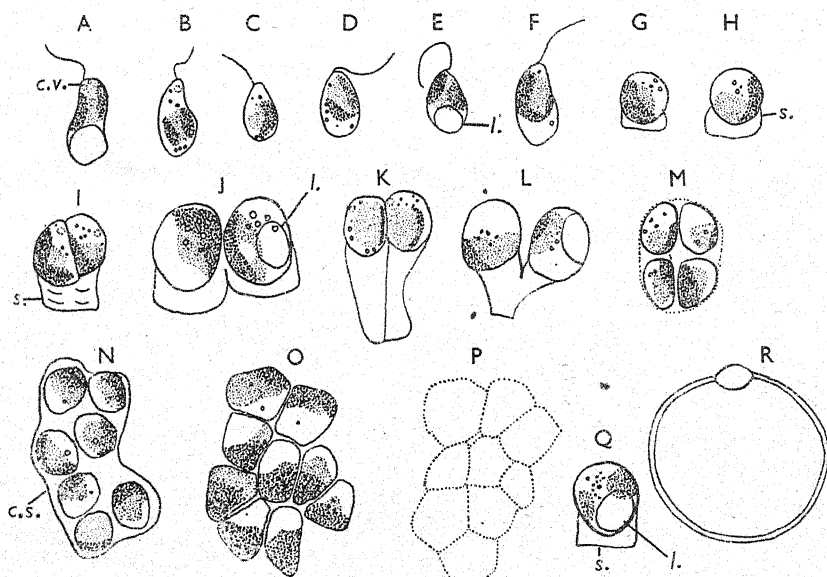


Fig. 2. *Chromulina aerophila* n.sp., forma B. A-F, motile cells; G-H, non-motile stages from surface film; I-L, division stages; M, four cells with a common stalk; N, formation of false palmelloid colony by fusion of the stalks; O, flattening of cell outlines due to crowding; P, empty cells as in O; Q, R, cysts. c.v. contractile vacuoles; l. leucosin; s. stalk; c.s. confluent stalks. (A-I, K-Q,  $\times 1000$ ; J,  $\times 1300$ ; R,  $\times 2400$ .)

spherical, well-marked stalk (Fig. 2 H-L) of the non-motile cells (6.8-10.2 by 8.5-12.0  $\mu$ ) is wider than in the other form, and the cells are devoid of flagella so that motility is not immediately regained on submergence. Their chromatophore (Fig. 2 I-L) is generally cup-shaped, owing to the rounded shape of the cell. The confluence of the envelopes described for forma A is more marked in the crowded cultures enriched with Benecke's solution, even the stalks of adjacent cells being confluent (Fig. 2 N) and becoming angular as a result of mutual pressure (Fig. 2 O, P). The whole pellicle thus appears as a palmelloid colony. Division, as in forma A, is restricted to these non-motile stages, and one finds two (Fig. 2 J-L; very rarely four, Fig. 2 M) cells with a common stalk.

The abundant growth of this form in cultures enriched with inorganic salts supports the view that it is truly holophytic (see *C. Rosanoffii*, p. 279). When the cells are loosely aggregated, it shows a similar iridescence and has the same capacity for orientation to the

light as forma A, but these features are lost when the cells are crowded. This lends support to the view that reorientation is due to movement of the protoplast as a whole. Cysts like those of forma A are very rare.

Although the motile cells are altogether like a *Chromulina*, the non-motile stage is dominant under certain conditions. In view of its unstable nature (especially in forma A), however, the alga is best regarded as a species of *Chromulina*. This point of view is supported by the life-history of *C. ferrea* (pp. 280-1), as well as by that of the three species, *C. Rosanoffii* Bütschli, *C. smaragdina* Gicklhorn, and *C. Woroniniana* Fisch, the first and last of which have much in common with *C. aerophila*. Certain differences and the absence of transitional forms stand in the way of referring all three to *C. Rosanoffii*. A critical consideration of the life-histories of these algae shows that the descriptions of them are in part incorrect.

*C. Rosanoffii* Bütschli (*Chromophyton Rosanoffii* Woron.), first discovered in Finland and in the St Petersburg Botanic Gardens (Woronin, 1880; Gaidukov, 1900), was subsequently recorded from tanks and aquaria in Germany (Wille, 1882, 1887; Ulehl, 1911), Czechoslovakia (Molisch, 1901) and this country (Fritsch & West 1927); Pascher (1913) states that it is very widespread. Its evident preference for small, artificial bodies of water is no doubt related to the existence of an undisturbed surface film allowing of the occurrence of the non-motile stage, which is essentially similar to that of *C. aerophila*.

Woronin (1880) found it in summer as a yellow or brown dust which consisted of a number of bubbles, spherical when small, vermiform when large, on the surface of the water of moorland pools. This coating disappeared during rainfall, but reappeared in fine weather. The bubbles included one or more spherical *Chromulina* cells which became motile when submerged; the motile cells (8-9 by 4-6  $\mu$ ) contained a single parietal chromatophore, one or two anterior contractile vacuoles and were devoid of a stigma. Under a coverslip they showed strong positive phototaxis, but when the drop was uncovered they bored their way through the surface film just as if it were a solid membrane. In passing through the film a mucilaginous envelope and a basal mucilaginous stalk are excreted, the former dissolving instantaneously on contact with water. The larger 'bubbles' are formed by the confluence of the mucilage envelopes around neighbouring cells.

If the surface film remained stable for some time, each cell divided into eight daughter cells within the mucilage envelope. The supposed winter stage of *C. Rosanoffii*, described by Woronin as living in the leaves of aquatic mosses (especially *Sphagnum*), may well belong to another species; nothing comparable to it has been observed in *C. aerophila*. Gaidukov (1900) grew the alga in a 0.1% Knop solution and thus showed that it could lead a purely holophytic existence, while Molisch (1901) studied its iridescence.

It is obvious that this alga is very like *C. aerophila*, and there can be little doubt that the envelope around the non-motile cells is again water. Woronin was altogether unable to observe the 'mucilage' when the cell was in contact with water and the statement that the stalk consists of mucilage cannot be altogether correct, since a portion of it must be incapable of being wetted (p. 275) if the cell is to remain above the surface film. The only clear difference between *C. Rosanoffii* and *C. aerophila* is thus the existence of eight-celled division stages in the former. Woronin's semi-diagrammatic figures of these stages are not wholly convincing, but *C. ferrea* n.sp. (pp. 280-1) shows that they may occur.

A misreading of Woronin's account has resulted in his figures of the naked stalked cells



and the eight-celled division stages being described as cysts and as products of germination respectively (Pascher, 1913, and all subsequent publications), but it is clear from Woronin's summary that these cells are not cysts, but division stages. He records (pp. 644-6) undoubted Chrysophycean cysts which are submerged and devoid of a stalk, in the questionable winter-form.

*C. Woroniniana* Fisch (1885), discovered in an aquarium at Erlangen, has a motile stage ( $6-8\mu$ ) very similar to that of *C. Rosanoffii*, while the coating formed by the non-motile stage shows a similar iridescence and is composed of rounded cells surrounded by a zone of hyaline mucilage and delimited from one another by fine lines. According to Fisch there is no stalk, and division of the cells was not observed. When *Sphagnum* was added, cysts were found in the hyaline cells. This species is very similar to the form of *C. aerophila* inhabiting acid water (forma B). The absence of a stalk is doubtful, since Fisch does not describe the cells in side-view. The surrounding 'mucilage' is probably the material of the stalk, which is short and wide as commonly in the form of *C. aerophila* just referred to (Fig. 2 I, J), while the fine boundary lines between the cells probably mark the points of juncture of the stalks (cf. Fig. 2 P).

In *C. smaragdina* Gicklhorn (1922)\* the non-motile cells are provided with a 'mucilage' stalk, which may sometimes be nothing more than a slight bulge on the cell membrane. The basal parts of the cells are surrounded by brown mucilage encrusted with ferric oxide (cf. *C. ferrea*). The cells divide repeatedly, therein resembling *C. Rosanoffii*. The daughter cells are either motile or non-motile; the former possess two green chromatophores, while the latter contain from four to ten green chromatophores and show an emerald iridescence. The green colour may be due to the abundant humus present, since accompanying individuals of *Mallomonas acaroides* were also green; transference to pure water, however, produced no change in colour. Gicklhorn refers to the non-motile stages as 'cysts', but they have none of the characteristics of Chrysophycean cysts.

## 2. *Chromulina ferrea* n.sp. (Fig. 3)

The motile cells ( $4.3-5.1$  by  $6.8-11.9\mu$ ) (Fig. 3 A-G) are markedly metabolic, especially in the posterior region which may taper to a fine point (Fig. 3 D, E). The flagellum reaches  $1\frac{1}{2}$  times the length of the cell, but its relative length depends on the shape of the cell. The two anterior contractile vacuoles pulsate alternately and one can occasionally distinguish smaller ones which fuse to form the principal ones. There is no stigma. The single parietal chromatophore is either a small curved disk (Fig. 3 C, G) or a band- (Fig. 3 A) or cup-shaped structure (Fig. 3 B, D).

The non-motile stage arises, as in *C. aerophila*, at the surface of the water, where it forms a brown scum. The cells develop a cup-shaped stalk which encloses them and their division products (Fig. 3 H-X) and generally consists of two parts, joined to one another; the basal submerged part is capable of being wetted, but not the part above. At the level of the surface film the stalk expands horizontally into a hoop-like structure (Fig. 3 J-O), which in surface view often appears surrounded by a wider layer of indefinite shape and rests on or in the surrounding water film (Fig. 3 J-L, O-R, f.); this layer occasionally bears small algal epiphytes on its under side (Fig. 3 K). The expansion of the stalk at the water surface may well help in maintaining the cells in an upright position.

\* Since writing this, in August 1940, I observed an alga agreeing with Gicklhorn's description of this species in the drain where, in previous years, *Chromulina aerophila* occurred.

Above the surface film the stalk continues for some way round the cell, immediately contiguous to the surface of the protoplast. Sometimes it seems to extend over almost the whole surface, leaving only a narrow apical pore. When such cells are submerged, the protoplast escapes by creeping through the pore (Fig. 3 X). The stalks are coloured brown through impregnation with ferric oxide, and when present in quantity render the community clearly visible to the naked eye. The non-motile stages of this species have not been examined in detail, but there appears to be complete similarity with *C. aerophila* and water is doubtless drawn up through the stalk in the same manner. There is, however, no iridescence. In the iron incrustation, the alga shows some resemblance to *Chrysotilos ferrea* Pascher (1931b).

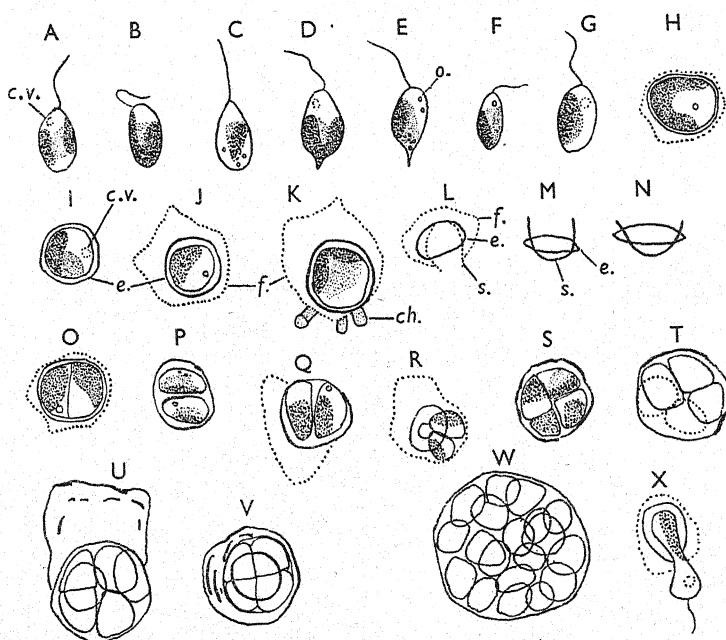


Fig. 3. *Chromulina ferrea* n.sp. A-G, motile cells; H-W, non-motile stages; L-N show the structure of the stalk; O-W, division of the protoplast; U, in side-, R in partial side-view; X shows escape of a single cell from the envelope formed by the upper part of the stalk. *ch.* *Chamaesiphon incrustans*; *c.v.* contractile vacuole; *e.* ring-like expansion of stalk (*s.*); *f.* irregular outer part of expansion of stalk on the surface film; *o.* oil. (All  $\times 830$ .)

A light breeze or rainfall are sufficient to cause submergence, followed by resumption of motility. Division will, however, frequently not have reached completion, and when this is so motility is not immediately resumed on submergence, since the flagella are still absent. In laboratory cultures, in which a stable surface film may persist for indefinite periods, all stages in division were invariably found and motile cells were also frequently present. It seems probable that, when division stages reach a certain size, they become top-heavy and fall over, thus becoming submerged. Cysts were not observed.

*C. ferrea* occurs during summer and early autumn in the shallow upper-littoral areas of the Pen Ponds, rich in humus derived from decaying leaves. It is never found, however, until the latter have become converted into a fine brown silt; at this stage the surface film is frequently partially covered with ferric oxide. The pH is usually 6.5-7.5, and, owing to the shallow water, the day temperature is often high (up to 31° C.). In the

laboratory the alga appeared in distilled water cultures of the same deposits, but not until the vegetable detritus is converted into a brown mud. If the deposit becomes acid (pH 4.0-6.0), *C. aerophila* takes the place of this species. The fact that *C. ferrea* is not a shade form perhaps accounts for the lack of a capacity for orientation to the direction of the light.

3. *Chromulina sporangifera* n.sp. (Fig. 4)

This alga, present in the Leg-of-Mutton Pond from February to April, has ellipsoid motile cells (6.8-10.2 by 2.5-3.4  $\mu$ ) showing little metaboly; the anterior end is rounded or slightly flattened and often oblique (Fig. 4 A, B). The flagellum is 1-1½ times the length of the cell (10.2-13.6  $\mu$ ) and there is no stigma. The band-shaped chromatophore has a slightly spiral trend (Fig. 4 A, B). The motile cells, like those of *C. ovalis* Klebs (also occurring in this pond), often colour the water brown.

When division is to take place, the movements become sluggish and the individual comes to rest among the detritus surrounding the stems and leaves of the aquatic macro-

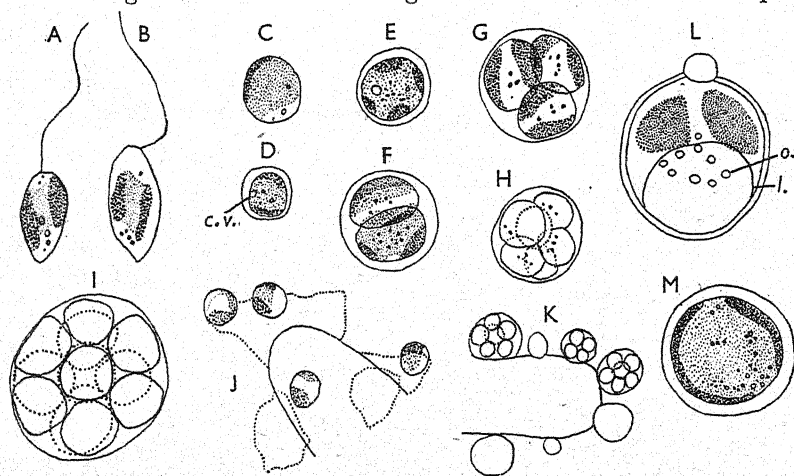


Fig. 4. *Chromulina sporangifera* n.sp. A, B, motile and C-E non-motile cells; E-I, sporangium-formation; J, K, cells and sporangia attached to vegetable detritus; L, cyst; M, young stage of same. c.v. contractile vacuoles; l. leucosin; o. oil. (A, B,  $\times 1600$ ; C, F, G, I,  $\times 1300$ ; D, E, H,  $\times 800$ ; J,  $\times 480$ ; K,  $\times 400$ ; L,  $\times 2650$ ; M,  $\times 1750$ .)

phytes. The protoplast assumes a spherical shape (Fig. 4 C) and increases in size, while a wide and firm mucilaginous membrane is secreted around it. The chromatophore extends over most of the periphery (Fig. 4 C-E) and may divide into two to four portions. Within this sporangium repeated division takes place (Fig. 4 F-I), so that forty or more closely packed daughter cells are formed. When liberated they are spherical, but they soon become actively motile and assume the ellipsoid shape. Contractile vacuoles are often observable in the non-motile stages (Fig. 4 D) as well as in the motile cells.

The production of cysts has not been observed, but huge numbers of them occur among the sporangia and on the bottom during the later stages of the period of abundance of this alga. The absence of other cyst-producing Chrysophyceae algae and the constant appearance of the cysts at a definite time leave, in my opinion, no doubt that they belong to *C. sporangifera*. They are commonly spherical (10.2-13.7  $\mu$ ) (see, however, Fig. 4 L) and have a smooth wall, the plug being often almost flush with the surface. They contain two chromatophores, as well as oil and leucosin.

The motile stages of this alga differ from those of *C. nebulosa* Cienk only in size, but the probable cysts are quite different. The production of sporangia, moreover, clearly differentiates the species from *C. nebulosa* and other members of the genus. *C. sporangifera* is found during the highly acid phase (pH usually 4.4-4.8) above the marginal sediments in the Leg-of-Mutton Pond, though completely lacking in the open surface waters (plankton). *C. nebulosa*, which occurs in moorlands, probably also inhabits acid waters (Pascher, 1913).

4. *Kephyrion littorale* n.sp. (Fig. 5 A-I)

The frequently brown-coloured envelope (6.0-6.8 by 4.8-5.1  $\mu$ ) is generally somewhat narrowed posteriorly and anteriorly (cf. however, Fig. 5 F), while it is more or less prominently thickened near the anterior end. The flagellum is approximately the same length as the naked protoplast and is inserted laterally, usually close to the stigma (Fig. 5 D, E). The two (rarely three, Fig. 5 F) parietal discoid chromatophores lie on the

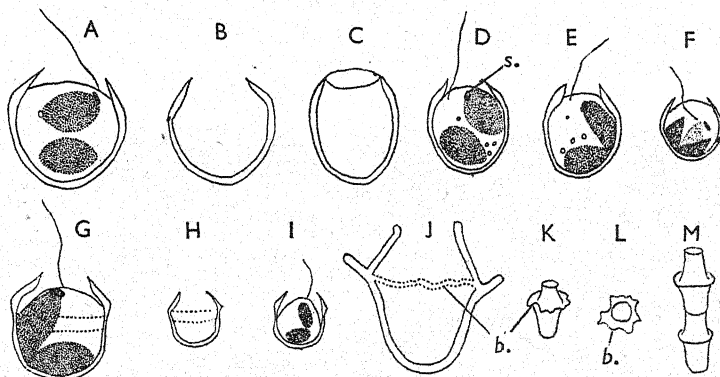


Fig. 5. A-F, *Kephyrion littorale* n.sp.; G-I, *K. littorale* var. *constricta* n.var.; J-M, *Stenocalyx monilifera* Schmid; J, original; K-M, after Schmid. b. collar; s. stigma. (A-C, G, J,  $\times 2400$ ; D, E,  $\times 1400$ ; F, H, I,  $\times 800$ ; K-M, magnification not given (circa 800?).)

same side of the cell, commonly so closely applied to one another that they appear as a single V-shaped structure (Fig. 5 E); the stigma usually lies at the tip of the anterior chromatophore. Oil is present, but leucosin has not been observed. The protoplasts can escape from the envelope as free-swimming individuals, but no other stages have been observed. Cysts are unknown in this genus.

In certain individuals (var. *constricta* n.var., Fig. 5 G-I) the envelope (5.1-6.0 by 5.1  $\mu$ ) has a rounded and generally slightly flattened base (Fig. 5 H), while the almost parallel sides are constricted in the middle (Fig. 5 G). The thickened anterior edges taper to a sharp point. Both the type and the variety occurred in the littoral plankton of the Pen Ponds, especially during the low temperatures of winter when the water was somewhat acid (pH 6.3-6.9).

*Kephyrion littorale* differs from other species of the genus in possessing more than one chromatophore; it is also distinguished by its well-marked stigma, with its characteristic position. A stigma is recorded in *K. mastigophorum* Schmid (1934), while Conrad's figures of *K. cupuliforme* (Conrad, 1930) suggest the presence of a small stigma. These two species appear to be those most nearly related to *K. littorale*.

One species (*Pseudokephyrion depressum* Schmid (1934)) of the closely related genus



*Pseudokephyrion* (Pascher, 1913), which only differs in the presence of two unequal flagella, shows a constricted envelope similar to that of *Kephyrion littorale*. Further, *Kephyriopsis ovum* Pascher & Ruttner (Pascher, 1913), which lacks such a constriction, shows a similar anterior thickening of the envelope tapering at its tip. Even after staining and careful observation under high powers, the cells of *K. littorale*, however, display only one flagellum. There seems little doubt that the three genera mentioned are closely related, and the discovery of *Ochromallomonas* (p. 286), the presence of unequal flagella in *Synura uvella* (p. 288) and the marked similarity of many forms of *Chromulina* and *Ochromonas* (Fritsch, 1935) render it more than likely that the presence of one or of two unequal flagella is not a very fundamental difference in this class.

5. *Stenocalyx monilifera* Schmid (Fig. 5 J-M)

This was of very rare occurrence in the plankton of the Upper Pen Pond in December and January 1937-8, and only one individual could be drawn; the remaining figures are taken from Schmid's original account (1934). The envelope (8.5-9.5 by 6.5  $\mu$ ), which has the shape of an inverted hat, is provided with a broad but thin collar a little above the middle (Fig. 5 J); in the Richmond material it has a slightly undulating course, as also in some of Schmid's specimens (Fig. 5 K, L). According to him (1934, p. 169) the collar is interrupted at one to three points, but this does not appear to be so in the Richmond material. There is a single parietal chromatophore and the flagellum is somewhat longer than the cell.

MALLOMONAS

1. *Mallomonas heterospina* n.sp. (Fig. 6 A-D)

The oval cells (12-15 by 7-8  $\mu$ ) possess a flagellum of about the length of the cell. There is no stigma. The two parietal chromatophores are separated from one another by only a narrow area, and a large mass of leucosin lies in the posterior half of the cell (Fig. 6 B). The species is distinctive, in that the silicified scales bear spines of two different types, simple and needle-shaped ones at and around the front end and such as are provided with a C-shaped hook, inserted just below the apex, on the posterior half or two-thirds of the body (Fig. 6 D). This species was present in small numbers in the Clay Pit between January and March.

2. *Mallomonas intermedia* Kisselew (Fig. 7)

This species occurred in the plankton of the Clay Pit and the Pen Ponds during the winter. The cells (20.5-28.9 by 8.5-15.3  $\mu$ ) are generally widely oval, often rounded posteriorly and more pointed anteriorly (Fig. 7 A). The flagellum is approximately the length of the cell. There are several small anterior contractile vacuoles which unite to form two or three larger ones. The large spherical nucleus is usually situated in the posterior half of the cell, while there are two parietal chromatophores. Oil and leucosin occur. Kisselew (1931) describes *M. intermedia* as having only one chromatophore, but his figure is not convincing; when the two chromatophores overlap markedly, there may appear to be only one on superficial observation.

The oval scales (6.0-6.8 by 3.4  $\mu$ ) are arranged in oblique transverse series (Fig. 7 C) and exhibit a circular depression in front and a V-shaped thickening behind (Fig. 7 F, G). The long needles (28.9-34.0  $\mu$ ), which are sometimes slightly curved, have a flattened tip (Fig. 7 D, E), while the base is slightly thicker than the rest and sharply curved at its

point of insertion on the scale. This basal piece lies in the anterior depression of the scale (Fig. 7 D). The spines are orientated more or less radially (Fig. 7 A), although sometimes all of them are directed backwards (Fig. 7 B). The almost spherical cysts (17.0-18.7 by 15.3-17.0  $\mu$ ) are smooth and possess a small pore (Fig. 7 H, I) occupied by a rounded plug.

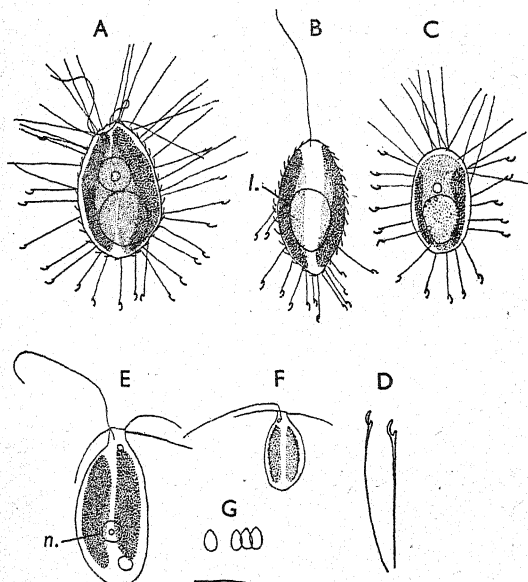


Fig. 6

Fig. 6. A-D, *Mallomonas heterospina* n.sp. A-C, motile individuals; in B the spines on the anterior half have been lost; D, spines from the posterior region of the cell; E-G, *M. limnicola* n.sp.; G, spines and scales. *l.* leucosin; *n.* nucleus. (A-C, F, G,  $\times 825$ ; D, E,  $\times 1650$ .)

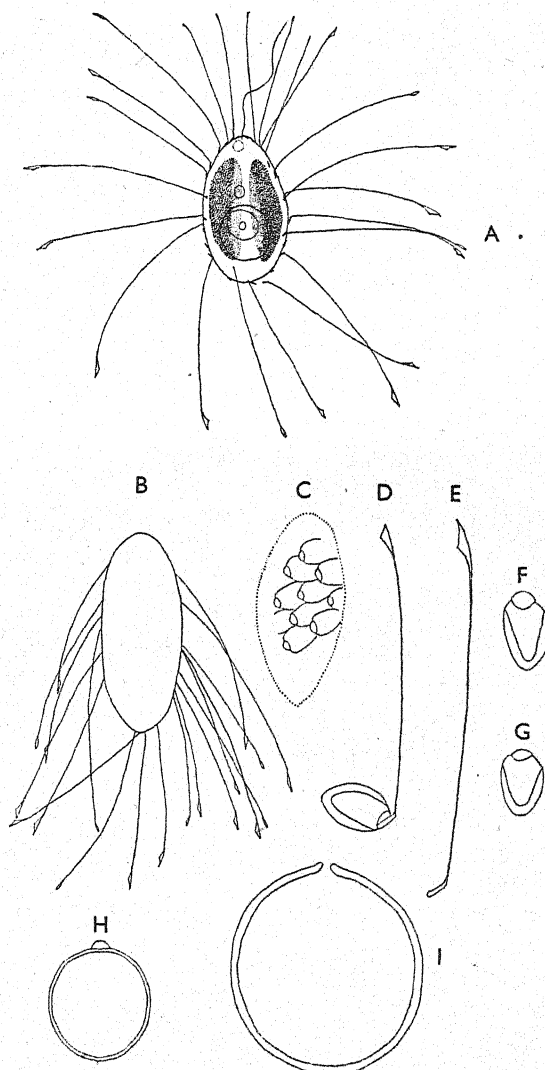


Fig. 7

Fig. 7. *Mallomonas intermedia* Kisselew. A, B, motile individuals, B with backwardly directed spines; C, scales showing oblique transverse arrangement relative to the long axis; D, scale with attached needle; E, needle; F, G, scales; H, I, cysts. (A-C, H,  $\times 780$ ; D-G, I,  $\times 1550$ .)

The individuals do not agree wholly with Kisselew's (1931) description of *M. intermedia* and show some likeness to *M. acaroides* Perty (Krieger, 1930; Conrad, 1933). Conrad has expressed the opinion that the former species is actually identical with *M. acaroides*, but this is improbable, for the reasons given below. The needles of *M. acaroides* are recurved, with a lancet-shaped tip, while those of *M. intermedia* are as above described. In *M. acaroides* the V-shaped marking on the scales is caused by punctae (Conrad, 1933) and finer punctae occur all over their surface, but no such

structure is described for *M. intermedia*, nor is it evident in my material, even when mounted in styrax or  $\alpha$ -monobromonaphthalene. The cysts of *M. intermedia*, here described for the first time, differ from those of *M. acaroides* in their smooth wall. Lastly, *M. acaroides* is a summer plankton form, while *M. intermedia* is a winter plankton form.

3. *Mallomonas limnicola* n.sp. (Fig. 6 E-G)

The oval cells (11.9-17.1 by 6.0-6.8  $\mu$ ), which are commonly more flattened on one side than the other (Fig. 6 E), have a rounded posterior end, while the anterior is drawn out into a short collar from the base of which the flagellum (ca.  $1\frac{1}{2}$  times the length of the cell) arises. There is no stigma. The nucleus (Fig. 6 E, n.) is located posteriorly and there are two parietal chromatophores placed opposite one another. Oil is present, but no leucosin was observed. The scales (3.0 by 1.5  $\mu$ ) are oval, with the anterior end more pointed than the posterior (Fig. 6 G). The two or three needles, which are attached to the scales of the collar, are slightly thicker at the base.

This species was found in small numbers close to the bottom deposit in the marginal regions of the Pen Ponds during the summer and autumn.

*OCHROMALLOMONAS* n.gen.

*Ochromallomonas pelophila* n.sp. (Fig. 8 A-H)

The motile unicellular individuals are usually oval to ellipsoid in outline, with a blunt anterior and a more or less pointed posterior end (Fig. 8 A, B). The shape varies in correspondence with the slight metaboly of the posterior end. The two flagella are of different lengths and inserted a little way behind the anterior end; the longer is about twice the length of the shorter and always somewhat longer than the body of the cell. Size of cell and length of flagella vary considerably, but there is a definite relation between the two; the cells are 17.5-32.0 by 8.5-14.0  $\mu$ , the longer flagellum 23.0-38  $\mu$ , the shorter 10.5-19  $\mu$  long. The longer flagellum exhibits an undulatory movement along almost the whole of its length, while the shorter stands out stiffly (Fig. 8 A, B) with the tip actively vibrating, like the lash of a whip. The organism swims slowly with a gliding motion.

The periplast is covered with numerous overlapping oval scales (Fig. 8 G, H), which

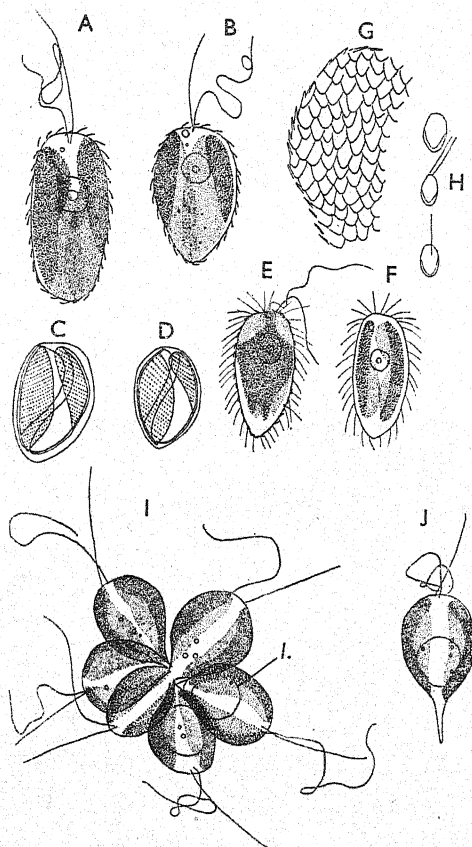


Fig. 8. A-H, *Ochromallomonas pelophila* n.gen., n.sp.; A, B, E, F, motile individuals; C, D, semi-diagrammatic, to show the form of the chromatophores (lobes nearest the upper surface shaded); G, surface with scales; H, scales and needles; I, J, *Synura uvella* Ehrenb.; J, cell isolated from colony. I, leucosin. (A, B, E, F,  $\times 1000$ ; C, D,  $\times 850$ ; G-J,  $\times 1350$ .)

give the cell a serrated contour. The V-shaped posterior end of each scale is thicker than the anterior portion (Fig. 8 H). The bristle-like needles attached to each scale project backwards and are extremely delicate, being often visible only under high power; it has not been possible to decide whether more than one needle is borne on a scale and whether they are attached at a definite point. The needles are probably largely non-siliceous, since they become more distinct after staining. They are easily broken off, and are almost invariably lost in preserved material.

There is no stigma. There are numerous posterior contractile vacuoles which frequently fuse to form two or three larger ones. The big nucleus with a conspicuous nucleolus (Fig. 8 A, B, F) lies in the anterior half of the cell. The two lobed chromatophores cover almost the whole of the periphery and overlap so that their exact limits are not always easily observed (Fig. 8 A-F). The two lobes of a chromatophore are orientated at an angle to one another so that they only partially overlap, but the free part covers to a greater or lesser degree the corresponding lobe of the other chromatophore (Fig. 8 B and C, D, which are semi-diagrammatic). As a result, the boundary between the two chromatophores appears as a narrow curved line at two different levels. The food reserves are oil and leucosin. No cysts have been observed.

This alga\* occurred in the infra-littoral regions just above the bottom-deposit of both Pen Ponds during summer and autumn as well as in small pools left in the moist mud along the edges. I have also found it once in a similar habitat at Roche Abbey, Yorkshire. It has only been observed where the bottom deposit is rich in humus.

The structure of *Ochromallomonas* is similar to that of *Mallomonas*, except for the presence of two unequal flagella. This places it, on the present classification of the Chrysomonadales (Pascher, 1913; Fritsch, 1935), in the Ochromonadeae rather than in the Chromulineae to which *Mallomonas* is referred, although the flagella are less markedly unequal than in most Ochromonadeae. It has already been pointed out by Fritsch (1935) that the resemblances between certain species of *Ochromonas* and *Chromulina* make it doubtful whether too much stress should be laid on the type of flagellation (see also p. 284). The discovery of an organism with all the characteristics of a *Mallomonas* but possessed of two unequal flagella lends further support to this view.

#### OCHROMONAS

A member of this genus occurred in cultures of deposits from the Leg-of-Mutton Pond enriched with Benecke solution. A few individuals, possibly belonging to the same species, have been met with in nature. The free-swimming individuals (6.8-10.2 by 5.1-6.8  $\mu$ ) are usually oval to ellipsoid and rounded at both ends, although the anterior end may show an emargination (Fig. 9 E). The long flagellum may be shorter than the cell or slightly longer, while the short one, often invisible in the living individuals, is from one-third to one-quarter the length of the longer. The pale yellow to greenish yellow band-shaped chromatophore occupies only part of the cell, with the stigma situated close to its anterior end. The single contractile vacuole is formed by the fusion of one or two smaller ones and, before evacuation, is sometimes so large as to distort the protoplast.

Nutrition, although presumably also holophytic, is very commonly holozoic, since the

\* Prof. Harris has kindly shown me rough drawings of a similar organism from the Reading district. It is doubtful whether it belongs to the same species.



majority of individuals contain one or more food vacuoles and ingest both bacteria and unicellular algae (Fig. 9 C, G-I, M), especially small cells of *Chlamydomonas* and *Chromulina*, some of which are larger than the *Ochromonas* itself. The frequent amoeboid stages (Fig. 9 J-L) retain their flagella, but exhibit slow movements with the help of blunt pseudopodia. Oil occurs as a reserve product. Leucosin has not been observed.

Occasional 'giant' cells (14 by 12  $\mu$ ) possess large food vacuoles containing several ingested organisms (Fig. 9 M) and are probably a consequence of excessive holozoic nutrition. In such forms the small pale chromatophore is sometimes scarcely distinguishable.

These specimens seem to be nearest to the form of *O. mutabilis* Klebs described by Doflein (1923) and differing from Klebs's (1893, p. 411) form in the presence of one instead of two chromatophores. Doflein is of the opinion that Klebs's record of two chromatophores in *O. mutabilis* is erroneous.

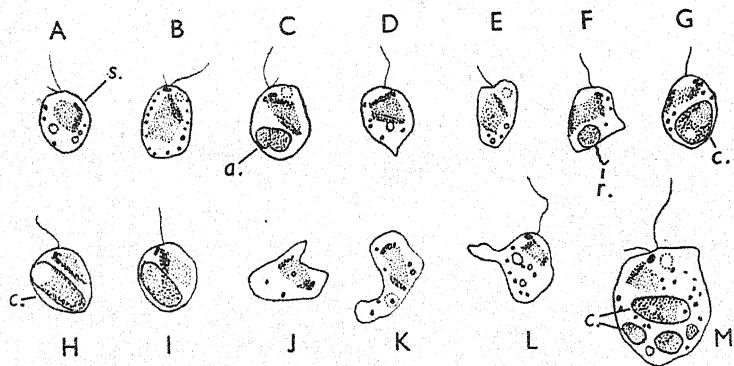


Fig. 9. *Ochromonas* sp. J-L, amoeboid stages; M, 'giant individual'. c. ingested *Chlamydomonas*-cells; r. rhizopodium; s. stigma. (All  $\times 1100$ .)

*Synura uvella* Ehrenb. (Fig. 8 I, J)

The variation in the structure of the skeleton of this common colonial alga has been abundantly described and has been used as a basis for distinguishing a number of species (Korschikoff, 1929). Such variations were observed also in my specimens, but have not been specially investigated. On the other hand, the differences between the two flagella were studied. The longer exhibits an undulatory motion, while the shorter is rigid and shows whip-like lashing movements. In fixed material the long flagellum usually appears much bent and twisted, while the short one remains straight (Fig. 8 I, J). The flagella thus resemble those of *Ochromallomonas* and of the *Ochromonadeae* generally, though the difference in size is not nearly so marked as in many of the members of this group.

*Synura* is generally classed among the Hymenomonadaceae (Pascher, 1913; Fritsch, 1935), a family characterized by the possession of two equal flagella. An examination of the literature, however, shows that the flagella have been variously described. In one of Bütschli's (1878) figures (T. 12, fig. 13a) both equal and unequal flagella are shown in the same colony, while another (Fig. 13d) depicts a cell with a long twisted flagellum and a short straight one, just as in my specimens. Stein (1878) states that there are two equal flagella (cf. also Pascher, 1910, 1913; Conrad, 1926, 1931), but in his figure, which has been extensively reproduced, certain cells appear to have flagella of slightly different lengths.

Petersen (1912) records flagella of unequal length and, using Löffler's stain, shows that the long flagellum bears two rows of hair-like appendages, a feature later (Petersen, 1929) demonstrated also in various other members of the Ochromonadeae. Fritsch (1935) points out that even if the appendages are artifacts resulting from the method of treatment, they clearly indicate a difference in structure between the two flagella. Schiller (1926, Fig. 10 *a, b*) shows the flagella unequal or almost equal, while in 1929 he described a genus *Synuropsis* differing from *Synura* only in the presence of two unequal flagella and of red cell contents (cf. also *Pseudosynura*, Kisselew, 1931). This genus has been criticized by Conrad (1931) since red droplets of haematochrome are known to occur in *Synura*, and it is very doubtful whether it is really distinct from *Synura*. Korschikoff (1929), in merging his genus *Skadovskiiella* in *Synura*, states that the record of two equal flagella is incorrect.

Despite its frequent occurrence, much uncertainty thus attaches to the flagella of *Synura*. The possibility that there are two forms differing in the flagella cannot be disregarded, but it appears more probable that there is but a single species which has unequal flagella; if this is so, *Synura* should be classed among the Ochromonadeae. Examination of *Synura* from diverse localities would probably clarify the position. The scales and vacuolar system of *Synura* (see Korschikoff, 1929) are comparable to those of *Mallomonas* and *Ochromallomonas*. A reinvestigation of *Chlorodesmus* and *Syncrypta* from this point of view seems advisable.

#### *APISTONEMA PYRENIGERUM* PASCHER

The base of a limestone wall flanking a small stream at Roche Abbey, near Rotherham, Yorks, was coloured dull brown by the rich growth of two members of Chrysophyceae, viz. *Apistonema pyrenigerum* Pascher and a species of *Gloeochrysis* which it has not been possible to study fully. The stratum, though damp, was not saturated with water. It is noteworthy that the habitat agrees almost exactly with that in which Pascher (1931*a*) found the alga. The *Gloeochrysis* was also abundant on the walls of the ruined Abbey. Various filamentous Myxophyceae and small pennate Diatoms were intermingled in the stratum on the limestone wall.

The *Apistonema* occurred for the most part in the form of separate spherical cells (12.8 to 14.4  $\mu$  wide) often showing stages in division, but there were also filamentous stages (Fig. 11); transitions between the two were observed (Fig. 10 H-L). In samples kept saturated in the laboratory filamentous stages were dominant, suggesting that the form assumed by the alga depends largely on the amount of water present.

The cells possess a well-marked wall which, when two or more are connected as a result of division or in the filamentous stages, is composed of two layers (Fig. 10 D-F). The outer layer, which holds the cells together, is more gelatinous than the inner and is especially distinct at the corners between contiguous cells of a filament. The filamentous stages are very irregular and the component cells are rather loosely joined together. Frequently a number of the rounded cells, typical of the unicellular state, are present near the centre (Fig. 11). The branches are short, with elongate end-cells (15-20  $\mu$  long), and do not all lie in one plane. Pascher (1931*a*) states that the branches sometimes adhere laterally, forming pseudoparenchymatous strata.

The cells commonly possess two parietal chromatophores situated opposite to one another, though sometimes a single one is present (Fig. 10 E, K, L). Apposed internally

to each chromatophore is a single naked pyrenoid, though this is frequently not recognizable. A number of leucosin balls and oil droplets of smaller size are generally present. Pascher (1931*a*) describes repeated division of the cell contents with the appearance of stigmata on the daughter protoplasts, though swimmers were not observed. I have not seen such stages, nor were cysts found. The only species of *Apistonema* hitherto recorded for Britain is the marine form described by Anand (1937). *Apistonema* parallels *Pleurococcus* among Chlorophyceae and *Monocilia* among Xanthophyceae, both in structure and in the terrestrial habitat.

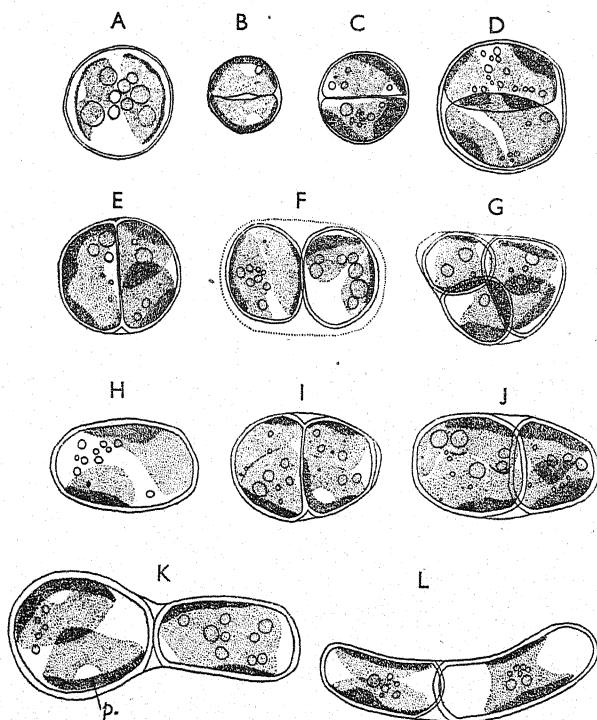


Fig. 10. *Apistonema pyrenigerum* Pascher. A, single spherical cell (coccoid state); B-F, stages in division; G, three daughter cells within mother-cell wall; H-J, transitions between coccoid and filamentous states; K, L, two-celled filaments. p. pyrenoid. (A, C, D-L,  $\times 1050$ ; B,  $\times 850$ .)

#### DIAGNOSES OF THE NEW FORMS

##### *Chromulina aerophila* n.sp.

Cellulae mobiles ellipsoideae vel ovaes, holophyticae,  $5-9\mu$  longae,  $4-5\mu$  latae, flagello circa tam longo quam cellula, vacuolis contractilibus duabus anterioribus, sine stigmatibus, chromatophora parietali lata taeniaeformi, plerumque cum pyrenoide: adeps et leucosin adsunt. Cellulae sedentes in pelliculam aquae superficiei, singulae vel aggregatae, plus minus sphaericae cum stipite posteriori. Divisio vel generatio cystarum per cellulas sedentes fiunt. Cystae membrana laevi obturamento paene plano chromatophoris 1-2,  $6-8\mu$  longae,  $8.5\mu$  latae.

*Chromulina ferrea* n.sp.

Cellulae mobiles ellipsoideae vel ovaes,  $4.3-5\mu$  longae,  $6.8-11.9\mu$  latae valde metabolicae, flagello  $\frac{3}{4}$  ad  $1\frac{1}{2}$ plo longiore quam cellula, vacuolis contractilibus duabus anterioribus, sine stigmatibus, chromatophora parietali magnitudinis variabilis. Cellulae sedentes super pelliculam aquae superficialem, stipite fusco brevi cupuliformi. Divisio per cellulas sedentes fit. Cystae ignotae.

*Chromulina sporangifera* n.sp.

Cellulae mobiles ellipsoideae,  $6.8-10.2\mu$  longae,  $2.5-3.4\mu$  latae, flagello  $1-1\frac{1}{2}$ plo longiore quam cellula, vacuolis contractilibus duabus anterioribus, sine stigmatibus, chromatophora taeniaeformi leniter spirali. Cellulae sedentes in materiam vegetabilem affixae. Divisio cellularum sedentium in forma sporangii partibus 40 vel pluribus. Cystae (?) sphaericae vel ovaes,  $10.2-13.7\mu$ , membrana laevi obturamento paene plano, chromatophoris duabus.

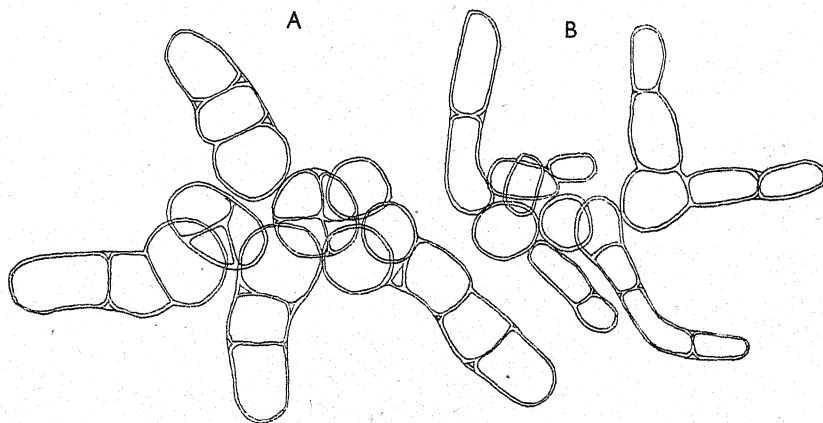


Fig. 11. *Apistonema pyrenigerum* Pascher. Filamentous stages. (A,  $\times 750$ ; B,  $\times 600$ .)

*Kephyrion littorale* n.sp.

Cellulae mobiles tegumento cupuliformi fine anteriore aperto inclusae,  $6.0-6.8\mu$  longae,  $4.8-5.1\mu$  latae, flagello circa tam longo quam cellula lateraliter prope stigma affixo, chromatophoris 2 (rare 3) parietalibus discoideis. Cystae ignotae.

var. *constricta* n.var.

Differt a typo tegumento dissimili,  $5.1-6.0\mu$  longo,  $5.1\mu$  lato.

*Mallomonas heterospina* n.sp.

Cellulae ovaes,  $12-15\mu$  longae,  $7-8\mu$  latae, flagello circa duplo longiore quam cellula, chromatophoris duabus parietalibus magnis; adeps et leucosin adsunt; squamae ovaes, aciculis anterioribus simplicibus, posterioribus uncinatis (in forma C). Cystae ignotae.

*Mallomonas limnicola* n.sp.

Cellulae ovaes,  $11.9-17.1\mu$  longae,  $6.0-6.8\mu$  latae, plerumque uno latere plus deplanato quam altero, flagello circa  $1\frac{1}{2}$ plo longiore quam cellula; squamae ovaes, aciculis 2-3 anterioribus in torque affixis. Cystae ignotae.

*Ochromallomonas* n.gen.

Cellulae mobiles, ovaes vel ellipsoideae, flagellis 2 anterioribus inaequalibus, uno



circa duplo longiore quam altero, chromatophoris duabus magnis leviter lobatis, stigmatate nullo, vacuolis posterioribus pluribus. Periplasta squamis imbricatis posteriore forma V decoratis oblecta, aciculis tenuibus simplicibus setiformibus. Adeps et leucosin adsunt. Cystae ignotae.

*Ochromallomonas pelophila* n.sp.

Vide *Ochromallomonas* n.gen. Cellulae 17.5–32.0  $\mu$  longae, 8.5–14.0  $\mu$  latae, flagellis uno 23.0–38.0  $\mu$ , altero 10.5–19.0  $\mu$ .

The author's grateful thanks are due to Prof. F. E. Fritsch for his help and criticism.

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## THE LIFE-CYCLE OF *STIGEOCLONIUM* *AMOENUM* KÜTZ.

By M. GODWARD

*Queen Mary College, London*

(With Plates 7 and 8, and 6 figures in the text)

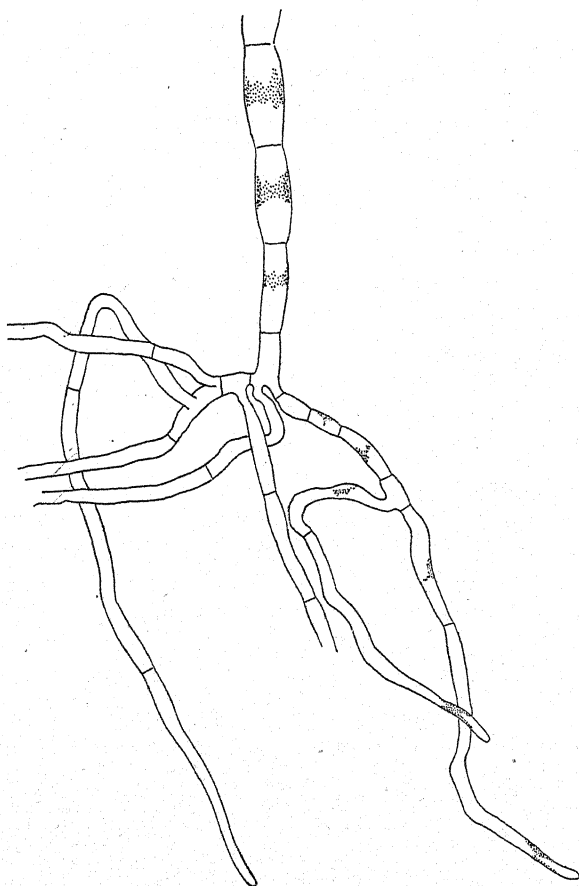
The material of *Stigeoclonium amoenum* upon which this account is based was found in the Pen Ponds, Richmond Park, attached to sand, stones, and plants along the edges, especially near the overflow. The plants occurred as isolated or aggregated tufts, 2 cm. or less in height.

The plant consists of a considerable branched erect system and a small rhizoidal attaching system (Text-fig. 1). The cells of the ultimate branches are a little longer to almost twice as long as broad ( $6-15\mu$  broad,  $15-21\mu$  long), the chloroplast occupying the whole length of the cell. The cells of the main axes are of two kinds: very short ones, about as long as broad, but slightly narrower than the others, and very long cells ( $6-15\mu$  broad, up to  $100-150\mu$  long). The short cells bear lateral branches which are frequently opposite (cf. Hazen, 1902). Hairs are not a feature of the plant when in a young and vigorous condition, but generally appear when vegetative growth is largely at an end (Pl. 7, fig. 1). Pl. 7, fig. 1 and Pl. 8, fig. 1 give an idea of the general appearance of the plant. Pl. 8, fig. 1 shows a strong resemblance to the figures of Hazen (1902) in respect of both the apical part of the thread and the axial and branching portions, but illustrates the somewhat unusual condition of single instead of paired branches; the latter are, however, clearly visible in Pl. 7, fig. 1. In Pl. 7, fig. 1 the long cells of the axes are divided up by zoospore partitions. The dimensions of the cells agree with those given by Hazen, while the young plant is like Gay's figure (Gay, 1891).

It has been thought advisable to give these details in view of the doubt which must always be felt in identifying a species of *Stigeoclonium* from vegetative characters only. There are several minor differences from the description given by Heering in the *Süsswasserflora*, viz. the ultimate appearance of hairs, the fact that the plant is normally attached, not free-floating; also the observation quoted by him, that erect threads may subsequently arise from the rhizoidal ones, has not been confirmed on this material.

Cultures on agar show that growth takes place diffusely throughout much of the length of the filament, the terminal cell also dividing. Cell division is rarer in the older parts. Cultures of material brought in from the natural habitat soon differed from the normal type; the differentiation of the cells in the main axes and the characteristic branching were obscured to a variable extent. On agar cells often divided parallel to the length of the filament (cf. Berthold, 1878, pl. XVII, fig. 7, *Chaetophora*). This may have been related to failure of the initiation of swarmer formation to go beyond the stage of nuclear division. Filaments divided up in this way broke up eventually into single rounded cells, the whole mass resembling a palmella without mucilage formation (Pl. 7, fig. 2). In this condition cultures survived some months longer than does the alga in the natural habitat.

In liquid cultures such stages were not produced and the plant died more quickly. Only plants arising from zygotes which had been produced in culture continued to grow satisfactorily in liquid culture for as long as a year or more. These plants were remarkably constant in morphological characters and closely resembled the natural growth. Exhaustion of the nutritive content of the medium caused the cessation of growth, the terminal cells first giving rise each to a hair.



Text-fig. 1. Rhizoidal prostrate system of plant grown in culture.

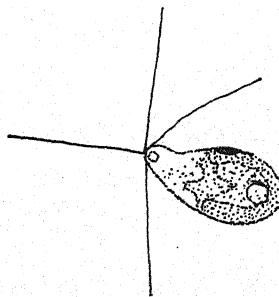
The culture solutions used included Benecke and Volvox solution (see Juller, 1937), but the most satisfactory solution was one prepared by Mr Chu of this department (Chu, 1942). Modifications of this solution were made in accordance with analyses of the water of Lake Windermere in Jan.-March, details of which were supplied by the Freshwater Biological Station at Wray. It was in this solution that a second generation of zygotes was obtained. Its composition is as follows:

$\text{NH}_4\text{Cl}$	0.00003 g./l.	$\text{Na}_2\text{SO}_4$	0.058 g./l.	$\text{FeCl}_3$	0.00003 g./l.
$\text{K}_2\text{HPO}_4$	0.028 g./l.	$\text{K}_2\text{SiO}_3$	0.0025 g./l.	$\text{KNO}_3$	0.25 g./l.
$\text{MgSO}_4$	0.08 g./l.	$\text{CaCO}_3$	0.01 g./l.		

together with soil extract or a small quantity of sterilized soil.  $\text{NH}_4\text{Cl}$  could be omitted and a higher concentration of  $\text{FeCl}_3$  was tolerated.

## THE PROCESS OF REPRODUCTION

Zoospores are readily produced when the material is brought in from the natural habitat or transferred to fresh culture solution. One or several zoospores may be produced from a cell. When more than one are produced, they are separated by fine partition walls (Pl. 7, fig. 1). The dimensions vary somewhat, depending on the size of the cell. They are less commonly produced from the axial cells. The zoospores (macrozoospores) are 4-flagellate,  $5-9\mu$  broad,  $8-10\mu$  long (Text-fig. 2). The eyespot is lateral, towards the middle of the body, as often recorded for other species. They always germinate by attaching themselves at the front end to a substratum, the body undergoing transverse division, and initially giving rise to an erect thread. The primary attachment is often by means of a small disk (cf. Fritsch, 1903; Juller, 1937). The part of the cell next to the substratum may from the first or later grow out into a pale or colourless rhizoid (cf. Gay, 1891, *S. amoenum*; Fritsch, 1903, *S. variable*). This subsequently undergoes a few divisions and small chloroplasts are formed. It branches (Text-fig. 1) and may be joined by other rhizoids growing down from cells of the thread above (cf. Fritsch, 1903, *S. variable*) so that a small basal attaching system is produced. Its cells are, however, always narrower, longer and paler than those of the erect system, and rather irregular in shape. The 'primary' rhizoids (growing from the zoospore which has settled down) and 'secondary' rhizoids (growing from other cells) are identical in appearance. This plant has therefore no prostrate system but only a rhizoidal attaching system. *Draparnaldia* and possibly other species of *Stigeoclonium* are similar in this respect (see p. 299). On drying agar during the summer all the cells of the filaments occasionally became transformed without change of shape into orange-coloured akinetes.



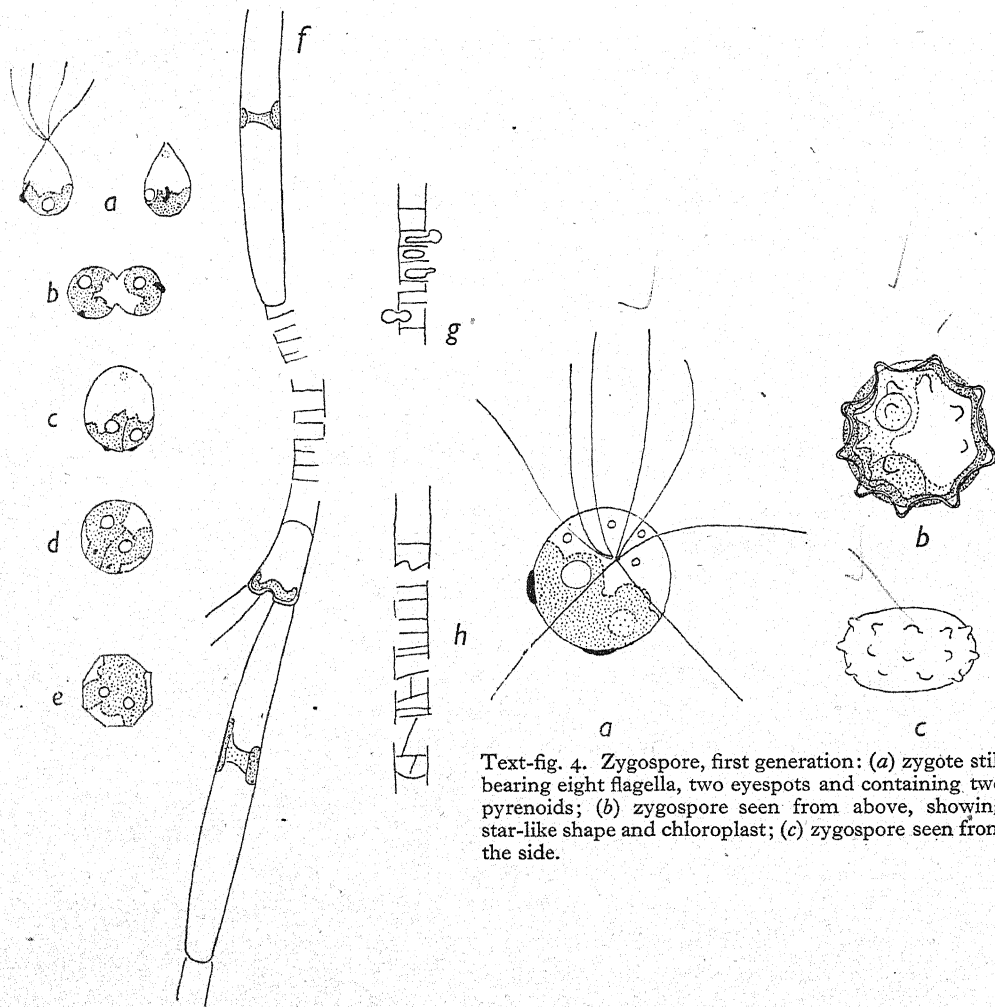
Text-fig. 2. Macrozoospore, showing mid-lateral position of eyespot.

The microzoospores (Text-fig. 3) are perfectly distinct from the ordinary zoospores, and behave as gametes. They are 4-flagellate,  $5\mu$  broad,  $7\mu$  long, with a projecting eyespot towards the back end of the body. They are slightly paler than the zoospores. The observations of Klebs and others on the relative positions of the eyespots in macro- and microzoospores, and of Klebs and Iwanoff on the stronger attraction to light, and longer period of motility of the microzoospores, have been confirmed in the course of this work. The period of motility of the microzoospores was prolonged for 12-24 hr. by keeping them in constant illumination.

Microzoospores are formed in all the cells by subdivision of the contents into an often large number of parts, which after their escape are seen to have been separated by fine partitions running in all directions relative to the long axis of the cell (Text-fig. 3, *h*) but most often transversely (Text-fig. 3, *f, g*; cf. also Berthold, 1878, pl. XV, fig. 3, of *S. variable*; cf. also plurilocular sporangium of *Pylaiella*). If the alga was brought into the laboratory and placed on agar for convenience of observation, large numbers of microzoospores were generally seen next morning. They generally continued motile for most of the day, beginning to copulate while others were still being released. Some were still motile on the second morning, but after two or three days all movement had ceased, the gametes having formed zygotes, although generally considerable numbers perished



without fusion. Most or all of the original material had been used up in their formation. Copulation occurs during movement (Text-fig. 3). The first stage is apparently entanglement of flagella after which there is lateral fusion beginning at the front end. The zygote when first formed is still motile, and is conspicuous by its large size, its two eyespots,



Text-fig. 4. Zygospore, first generation: (a) zygote still bearing eight flagella, two eyespots and containing two pyrenoids; (b) zygospore seen from above, showing star-like shape and chloroplast; (c) zygospore seen from the side.

Text-fig. 3. (a) microzoospores (gametes) showing projecting eyespot near back end of body; (b), (c), (d) stages of copulation; (e) young zygote with thin wall; (f), (g), (h) filaments which have produced gametes showing partition walls which separated gametic individuals within the cells. Note especially the numerous partitions of the long cell in (f), and the vertical as well as transverse partitions in (h).

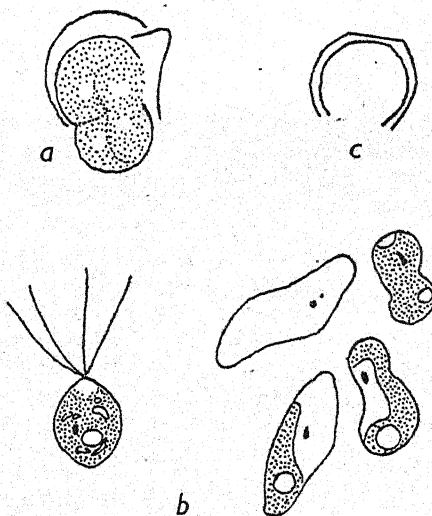
and eight flagella (Text-fig. 4). In from 10 min. to  $\frac{1}{2}$  hr. the flagella are lost, and the zygote rounds off, secreting a membrane which is at first smooth but soon becomes polygonal (Text-figs. 3, 4). Within a few hours the characteristic shape, to a casual glance that of a star, has been developed. When the zygote is turned on its side (Text-figs. 4, 6), it is seen that the star is of some thickness with convex upper and lower faces,

and rounded sides bearing irregular rows of short blunt spines. The chloroplasts and eyespots of the fused gametes remain distinct inside the zygote for less than a day; before they are completely mature only a single irregular chloroplast is to be seen and the eyespots have disappeared. Presently the green colour begins to fade, the contents becoming highly refractive and progressively more opaque. Finally, the colour becomes bright orange with shining contents probably due to oil (Pl. 8, fig. 2). The fact that the membrane is two-layered can be distinguished clearly only on germination. In liquid cultures most of the zygosporos float on the surface from the first, their shape being suited to flotation, and they form a bright conspicuous orange layer at the water level. It may be supposed that they are left on the soil at the margin of the pond, as the water-level rises in summer. The mature state was attained during June.

#### GERMINATION OF THE ZYGOTE

The zygotes remained in the living mature condition on agar, which was never allowed to dry completely, throughout the summer. Towards the end of the year the perfection of the stellate outline was somewhat lost and there was generally a reversion to the polygonal contour. Germination occurred during January or February if plentiful culture solution was supplied. The shape of the zygotes became more and more rounded, while there was an increase in size, the oil disappeared gradually and the contents slowly became green. Increase in size continued until the zygote became a smooth round object of nearly twice its original diameter. Division of the cell contents into four parts with eyespots then took place, after which a bulge usually appeared at one side (Text-fig. 5, *a*). At this stage the contents of the spore are still retained within an inner membrane though the outer membrane has broken. The inner membrane soon breaks also and four zoospores are suddenly expelled, sometimes becoming damaged in the process. The zoospores (Text-fig. 5, 6) in every respect resembled the macrozoospores of the ordinary filament, being  $9\mu$  broad and  $11\mu$  long, and they settle down and germinate in the same way as the macrozoospores. The germing develops into a plant (Pl. 8, fig. 1) like that which produced the gametes.

The remainder of the life-cycle was observed in a few cultures (grown from zygotes) which had been maintained from one spring to the next. The unnatural conditions of culture were probably the cause of sexual reproduction being delayed for a whole year, since there is no evidence that the plant exists in a filamentous condition in the habitat from June to December. In February microzoospores were produced in the cultures, but owing to their small numbers no good drawings could be obtained. The characteristic position of the eyespot was observed in them while in motion. Late stages in copulation

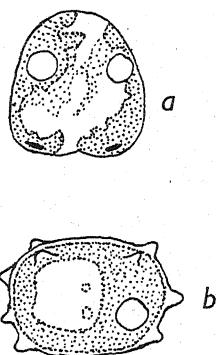


Text-fig. 5. Germination of zygosporos: (*a*) ruptured outer membrane, inner membrane bulging out with contents divided into four parts; (*b*) single zoosporos which has escaped from zygosporos; (*c*) membranes of empty zygosporos and the four zoospores which have escaped from it; from a culture on agar. The zoospores were unable to swim away before acquiring a wall.

were seen (Text-fig. 6, *a*), and the zygotes, though rather smaller and slightly more irregular in shape, developed all the characteristic features which had been observed in those of the first generation (Text-fig. 6; Pl. 8, fig. 3). Their further fate was not followed.

Four zoospores are the first product of germination of the zygospore. It is clear that the filaments arising from these four zoospores belong to the same phase of the life-cycle as the filaments collected from the natural habitat, since, like the latter, they give rise to gametes. Reduction therefore probably takes place in the first division in the zygospore, and the plant, with the exception of its zygote, is haploid throughout.

An attempt to confirm these conclusions by chromosome counts was not successful. The nucleus ( $2.6\mu$  broad) stained best with brasilin after osmic acid fixation. Prophase and metaphase stages of mitosis were obtained in material collected from the pond, provided it had not been in the laboratory for more than a day or two. The chromosomes were minute (less than  $\frac{1}{8}\mu$  long), and in the preparations obtained not sufficiently clearly defined to allow of photographic reproduction or an accurate count. The number of chromosomes was estimated at between 11 and 16. No clear mitotic figures were obtained from material kept in culture. The penetration of zygospores by fixative appeared to be slow. No evidence of meiosis was obtained.



Text-fig. 6. Zygospore, 2nd generation, in culture: (*a*) copulation stage; (*b*) zygospore, seen from the side.

#### PERIODICITY

In nature and to a great extent in culture this plant shows a remarkable and rigid periodicity (cf. *Ulothrix*) which may be summarized as follows:

- |                           |  |
|---------------------------|--|
| 1. Jan. 1936-9, Apr. 1940 | First appearance in habitat.   |
| (late spring)             |  |
| 2. Jan.-Apr.-May          | Production of zoospores.   |
| 3. Feb.-Apr.              | Production of zoospores and microzoospores.  |
| 4. Apr.-May               | Production of microzoospores only or mainly.   |
| 5. June-Dec.              | Absent from habitat. Difficult to keep alive in culture, the plant becoming abnormal or palmelloid, with the exception of those raised from zygotes. |
| 6. Jan.-Feb. 1938-40      | Zygotes germinated in culture.   |
| 7. Feb. 1940              | Production of gametes in culture, by plants raised from zygotes.   |

Attempts were made every year to induce gamete production and zygote germination out of season, without success. The methods tried included subjection to high and low temperatures, increased and reduced illumination, aeration, variations of culture solution by additions of glucose, vitamin C, auxins, soil extract, and increase and decrease of pH. Klebs induced the formation of microzoospores in *Stigeoclonium* by the addition of glucose, but these experiments were carried out in the spring, when their development may have been imminent in any case.

Some prolongation of the life of collected material during the summer months was secured by keeping it at a temperature of about 8° C. and under reduced light intensity.

Also germlings raised from zygotes in culture were eventually maintained throughout the summer in the laboratory, without morphological change. Even these plants, which are presumably physiologically adapted to culture conditions, produced their gametes in spring only.

I have also observed a spring maximum in Lake Windermere in a species believed to be *S. amoenum* although sexual reproduction was not noted.

Comparison with some of the better known species of *Stigeoclonium* is most easily made by means of the table. These species appear to fall into two groups, the first of which has a more fluid type of life-cycle than the second. In the first group occur biflagellate swarmers, as well as two kinds of quadriflagellate ones, and the microzoospores, may be bi- or quadriflagellate, and may or may not copulate. The star-shaped zygote is characteristic of the second group. In *S. amoenum* (second group), by contrast to *S. subspinosum* as described by Juller (first group), there is no diploid vegetative phase, no germination of the microzoospores without copulation, and the life-cycle is simple, like that of *Ulothrix*.

A point of interest is perhaps that in all these species the first germination stage of the macrozoospore, where this is known clearly, is an erect thread. The microzoospore, when not behaving as a gamete, similarly seems to germinate in a more or less constant manner, first forming a rounded cell which subsequently grows out on one or two sides into threads, at least one of which is often apparently prostrate, but there is not enough information on this last point for generalization.

A species, probably *S. farctum*, examined by the writer, gave rise to a quadriflagellate zoospore, which subsequently rounded off and grew out on opposite sides to form threads, one of which developed into the characteristic prostrate system of the species while the other was more or less arrested. This is in its first stage the same type of development as that of the microzoospore of group I in the table. It is just possible that this has some significance in relation to the life-history of *S. farctum*.

It would have been instructive if data on the development of the prostrate systems, if any, from the germlings arising from macro- and microzoospores could have been included in the table, but there are insufficient data. It is certain at least that in *S. amoenum* there is no real prostrate system; that from the base of the erect thread arises a rhizoidal system in no way different from secondary rhizoidal systems which may occasionally develop from other parts of the thread. The 'prostrate system' of *Draparnaldia* looks very much the same.

#### SUMMARY

The life-cycle of *Stigeoclonium amoenum* is described. There is a single vegetative phase which gives rise to gametes.

In germination the contents of the zygospor divide to four parts which escape as zoospores.

Germination of the zygote and production of gametes takes place in the early and late spring respectively.

A tabular comparison with life-cycles of other species of *Stigeoclonium* is given.

The author's thanks are due to Prof. F. E. Fritsch, F.R.S., who suggested the problem and edited most of the manuscript; to Dr J. W. G. Lund for the original collection of material, and to Dr R. W. Butcher for material of *Stigeoclonium* spp. including *S. farctum*.



Table I

Species	Author	Macro-zoospore	Germling of macro-zoospore	Inter-mediate swarmer	Micro-zoospore asexual	Germling of micro-zoospore	Micro-zoospore gamete	Zygo-spore	Product of germination of zygospore
1. <i>terzue</i>	Cienkowski 1876				Y from palmella				
"	Klebs 1896	Y	⊖		Y → ○ ○				
"	West 1904	Y	⊖				Y from filament	○	
"	Pascher 1907	Y	⊖			○ ← Y			
<i>longipilum</i>	Pascher 1907	Y	⊖	Y	Y → ○				
<i>nudiusculum</i>	Pascher 1906	Y				○ ← Y "dwarf germling"		○	
<i>pasticulare</i>	Pascher	Y	⊖		Y ← ○ ← Y "dwarf germling"				
<i>subspinosum</i>	Juller 1937	Y	⊖		Y → ○ ○		Y from palmella	○ → ⊖ → Y	2r.
2. <i>insigne</i>	Naegeli 1850		⊖						
	Treboux 1899						Y	☆	
<i>amoenum</i>	Godward 1941	Y	⊖				Y	☆ →	Y Y Y Y

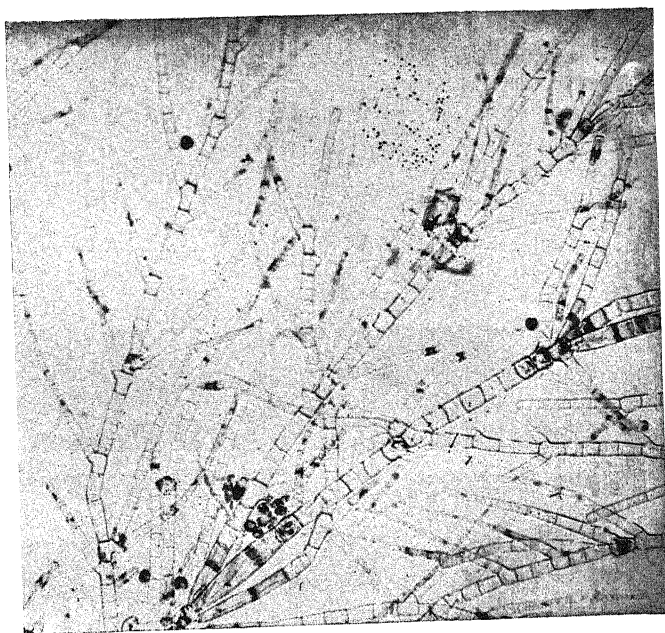


Fig. 1.

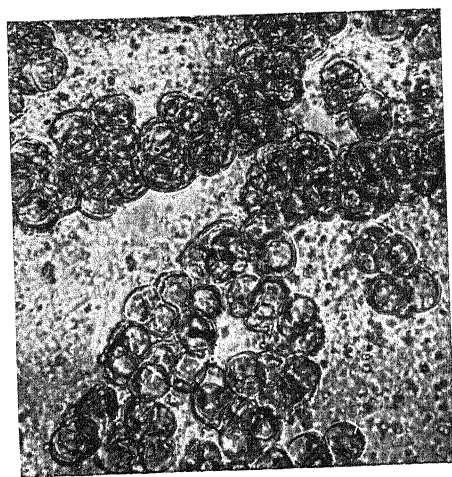


Fig. 2.

GODWARD—THE LIFE-CYCLE OF *STIGEOCLONIUM AMOENUM* KÜTZ.

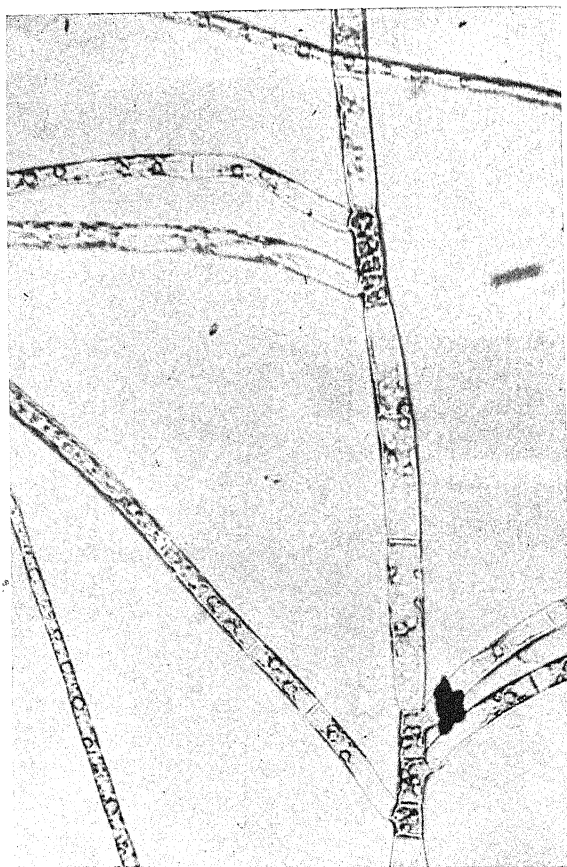


Fig. 1.

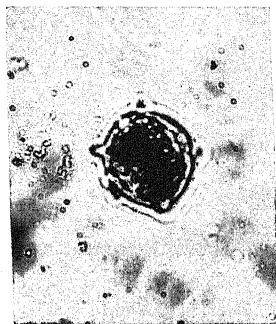


Fig. 2.

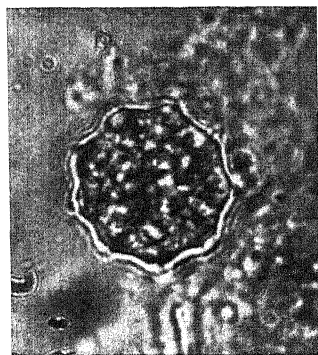


Fig. 3.

GODWARD—THE LIFE-CYCLE OF *STIGEOCLONIUM AMOENUM* KÜTZ.

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## EXPLANATION OF PLATES 7 AND 8

### PLATE 7

Fig. 1. Material from the habitat. Note small cells bearing opposite branches. Most of the long axial cells have become divided up by zoospore partitions but one or two undivided long cells with their narrow girdle chloroplasts may be seen.

Fig. 2. Palmella developing on agar. The course of the filaments from which the palmella arose may be traced.

### PLATE 8

Fig. 1. A plant developed from zygospore produced in culture, showing long and short types of axial cells, the short ones bearing lateral branches.

Fig. 2. Mature zygospore, first generation, with oil contents.

Fig. 3. Zygospore, second generation in culture.



## REVIEW

The Boylston Street Fish-weir: a study of the archaeology, biology and geology of a site on Boylston Street in the Back Bay District of Boston, Massachusetts. By FREDERICK JOHNSON and others. 7 × 10 in. Pp. 212, with 14 plates. Papers of the Robert S. Peabody Foundation for Archaeology, vol. 2. Andover, Mass.: Phillips Academy, 1942. Price \$2.00.

The past few years have witnessed a very determined attempt by North American scientists to lay down secure foundations for the prehistoric archaeology of their great continent: it has proved a task of considerable severity for suitable evidence, especially of a stratigraphic character, is infrequently found, and effective help by correlation with the great cultures of other continents can be expected to follow only in the most indirect way. We are going therefore in the next few decades to see a considerable assault made, and if so far advances are small, they nevertheless display the great initiative, imagination, and ability of the forces which will later achieve much.

The present paper concerns the discovery of a humanly-fashioned structure of sharpened stakes driven into tidal mud, and now revealed in building and subway excavations under many feet of silt in the Back Bay District of Boston, Mass. Acute observation and quick realization of the opportunity led to a very intensive investigation of the provenance of this discovery. Various specialists contribute information concerning the mineral sediments, foraminifera, mollusca, the oysters (which grew very freely on and among the stakes), the diatoms, the identity and chemistry of the wood of the stakes, pollen analysis of the silt and of an underlying peat (which predates the artefact), etc. The introduction, the description of the disposition, condition, and character of the artefact, and discussion of the results have been written by Frederick Johnson who directed the work throughout. This form of presentation has the happy effect of permitting the specialists to give free expression to their own views, whilst the tempered and restrained judgement of the senior author keeps before the reader the much narrower field of what the data actually prove.

A great many thousand narrow sharpened stakes were found driven into tidal silt; they formed several aggregates into bands (most of which ran N. and S.) but they were also present in dense clusters forming no evident pattern. Only a fragment of the whole original structure was present. It seemed that the staking had been added to as time progressed, and as silting buried the bases of the oldest stakes, and it appeared from their transgression of a sloping peat bed that the stakes were set to a tide-level. This is borne out by the growth upon them of mollusca. The upper ends of the stakes are now all rounded and at a very uniform level, more likely due to weathering and eroding at the air-water surface than to an original setting to this limit. Particularly towards the top and base of the staking there were disposed among the stakes loose horizontal masses of brush, referred to rather unfortunately as 'wattling'. From the title it will be apparent that the senior author favours the view that this is a fragment of a fish-weir, and in an appendix he describes some types of present-day American fish-weirs. It is agreed however that this conclusion is merely tentative, and it is concluded from the evidence of the oysters that it might possibly have served the purpose of collecting oyster-spat. The curious aggregations of stakes and brush in lines and masses much suggest to the reviewer methods used on East Anglian coasts and muddy estuaries for stabilizing tidal mud, but it seems unlikely that any save early white colonists would have used such a technique, and other evidence indicates that such colonists probably appeared long after this structure had been submerged and buried. All the same, one would like the assurance that among the thousands of stakes present, examination showed none to have the distinctive marks of cuts by metal axes on their sharpened bases.

From a careful investigation of the local stratigraphy an attempt is made to reconstruct the history of the silting up of this part of the estuary of the Charles River, but very conflicting views as to the physiography appear in the book itself. This is in part due to the restricted area about which information is available, and in part to the very inadequate state of our knowledge of exactly how deposition can go on above a submerging coast-line. The work demonstrates very clearly how vital it is to get a thorough and accurate determination of the 'peat' types encountered, if one is to deduce from them the conditions of deposition. In this instance the middle of the peat is acknowledged to be allochthonous, and the whole of the peaty layer has the character of a detritus

organic mud: this would explain its sloping nature and would modify the use made of this layer in deducing the relative position of land and sea-level when it was forming. Not only in America but in the established schools of Europe the advent of pollen-analysis has produced an undeserved and dangerous neglect of the technique of identification of peat types themselves.

It must also be borne in mind that at the landward end of tidal rivers fresh-water autochthonous peat can form as low as mean sea-level, and fresh-water detritus peat can be laid down still lower. This happens in East Anglia at this moment. Moreover, should such a tidal river have a long and winding course to the sea whilst its upper reaches (just unaffected by tidal rise and fall) lie close to a low coastal belt, there is always the danger of a sudden break-through during storms, so that salt water invades the top of the river-system. With no change in level therefore, many feet of brackish-water silt may be formed very quickly indeed above the fresh-water peats and lake-muds. This again has recently happened in East Anglia, at least so far as the initial breach and flooding with salt water. Such considerations as these materially affect the conclusions 'that the Fish-weir was used during a period of rising sea-level which began before the structure was built, and which may have lasted to the present-day', and 'that humans were living in the Charles River estuary at a time when the level of the sea, in relation to the land, was about fifteen feet eight inches lower than it is at the present time'. It is nevertheless fairly certain that a large relative rise in sea-level has occurred since the 'fish-weir' was constructed.

An attempt is naturally made to correlate this evidence of early human activity in the estuary, not only with sea-level changes, but with alterations of climate such as may be indicated by the remains of organisms present in the estuarine deposits. These seem to show that the climate when the lower peat formed was slightly warmer than it is now, and that during deposition of the silt conditions became cooler. Two intensive pollen-analyses also bear upon the question of climate evolution, the first, by W. S. Benninghoff, concerning the lower peat, and the second, by A. S. Knox, concerning the silt. Each author has employed a technique of pollen preparation unfamiliar to European workers: Benninghoff used the method of Barghoorn and Bailey of alternate treatment with strong chlorine water and hot sodium sulphite, and Knox used a method of his own devising of removing the pollen from the mineral sediments by flotation and centrifuging in a bromoform-acetone mixture with a specific gravity of 2.35. It is to be hoped that a critical account will be given of the effectiveness of this second technique in comparison with the standard HF treatment. It must be confessed that the results of this careful work are indecisive, for the pollen diagrams, beyond showing a forest composition like that of the same region to-day, are very featureless, and there is as yet no determined sequence of pollen zones for that part of the eastern U.S.A. against which they can be matched. It is very interesting to find that comparison of the pollen in the silt and peat shows none of the great over-representation of conifers which is the common and unexplained characteristic of estuarine silts round the North Sea coasts (cf. Brinkmann, Polak, Godwin and Clifford): indeed the similarity of pollen spectra from peat and silt almost warrants the neglect to consider the possibility of secondary pollen in the silts. Knox has ingeniously and boldly correlated the peat and silt diagrams with a long marsh profile taken three miles from the fish-weir site, and has in turn attempted a correlation with the European climatic periods. Although these attempted correlations are not much more than guesses, the data on which they are formed will stand ready for incorporation into the wider body of information which will before long define the course of post-glacial evolution of North America. We would suggest again the enormous value of detailed stratigraphic investigation of any peat lands which are being dealt with: careful working out of the lateral and vertical extension of different peat-layers will give far better basis for the recognition of dry and wet periods in the development of a lake or bog, than single profiles can ever do, and the Wellington Marsh series would gain enormously in value if it were backed by data of this kind. As it is, one would hesitate to accept the view that its basal layers indicate climatic dryness, and upon this turns the whole attempted linkage with European periods and the dating which is tentatively proposed.

More than ever it becomes apparent that these studies of the interaction of man and other organisms on the one hand, and of climate and physiographic agents on the other, fall into a field of 'palaeo-ecology' which can be most readily explored by learning and applying the lessons of ecological studies in comparable regions and habitats at the present day.

H. GODWIN.

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